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146. THE HEAT DENATURATION OF ALBUMIN AND GLOBULIN IN MILK

II. DENATURATION AND DEGRADATION OF PROTEIN AT TEMPERATURES OF 75–120° C.

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IN a previous paper(1) the literature on the denaturation of albumin and globulin that takes place when milk is heated was reviewed, and a series of data given for the rate of this denaturation at temperatures up to 75° C. The present paper deals with the extent to which albumin and globulin are denatured, and to which secondary proteins of a proteose-peptone nature and non-protein nitrogenous substances are produced, when milk is heated for various periods at temperatures of from 75 to 120° C.

EXPERIMENTAL

(1) *Heat treatment at* 75–100° C.

Portions of the milk samples were taken in narrow-necked 100 ml. flasks and raised rapidly to the temperatures required by immersion in a calcium chloride solution at 110° C. During the heating the milk was stirred vigorously. The flasks were then stoppered and transferred immediately to thermostats containing calcium chloride solution covered with a layer of oil. The flasks were shaken at intervals whilst in the thermostats, and after the required periods were removed and cooled in iced water.

The flasks were filled with milk to such a level that, on heating, the milk expanded just into the neck. This procedure reduced considerably the exposed surface. There was no "skin" formation, and evaporation from the milk was negligible.

(2) *Heat treatment at* 115 *and* 120° C.

100 ml. flasks were filled with milk as in (1), plugged with cotton-wool, and heated by steam under pressure in an autoclave. The flasks were weighed before and after heating, and a slight loss in weight made up with water.

Methods of analysis

The following direct determinations were made on the unheated and heated milk samples:

(*a*) *Total nitrogen.* 5 ml. of the milk were weighed in a 100 ml. graduated flask and diluted to the mark with water. 20 ml. of the diluted milk (=1 ml. of the original milk) were digested in a 200 ml. Kjeldahl flask with 5 ml. of

concentrated sulphuric acid, 2 g. of potassium sulphate, 0·2 g. of copper sulphate, and 2 drops of selenium oxychloride. Then the contents of the flask were diluted, made alkaline, and steam-distilled into excess of $N/70$ sulphuric acid, the excess acid being titrated with $N/70$ sodium hydroxide.

(*b*) *Soluble albumin, globulin, proteose*[1] *and non-protein nitrogen.* For the maximum precipitation of casein from the unheated samples, and of casein plus denatured albumin and globulin from the heated samples, 10 ml. of the milk were weighed in a 100 ml. graduated flask, and about 80 ml. of water at 40° C., and 1 ml. of 10 per cent acetic acid added. After 10–15 min. 1 ml. of normal sodium acetate was added and the contents of the flask allowed to cool. The volume was then made up to the 100 ml. mark with water, well mixed, and filtered on a dry paper into a dry flask. 20 ml. of the filtrate were digested for the determination of nitrogen content as in (*a*).

(*c*) *Non-protein nitrogen.* For the precipitation of total proteins, 10 ml. of milk were weighed in a 50 ml. graduated flask and diluted to the mark with 12·5 per cent trichloracetic acid solution. The contents of the flask were well mixed and, after 30 min., filtered on a dry paper into a dry flask. The nitrogen content of 20 ml. of the filtrate was determined as in (*a*).

Results

The results obtained by the above methods will be presented and interpreted in the light of the data concerning the composition of the soluble protein fraction of milk that are given and discussed on pp. 6–14. For each milk sample, the maximum amount of albumin and globulin that could be denatured by heating that sample will be considered to be the total true albumin and globulin content, and the remainder of the soluble protein to be of a proteose nature.

(1) In Table I are set out the essential results for four different milk samples which were treated as follows:

Milk sample (A), heated at 75, 80, and 90° C.
„ (B), „ 80, 90, and 95°·C.
„ (C), „ 95, and 100° C.
„ (D), „ 90, 95, and 100° C.

There was no change in the total nitrogen and non-protein nitrogen contents of any of these samples as a result of the heat treatment.

(2) In Table II are given the results for one sample of milk which was heated at 115 and 120° C.

[1] The term proteose will be used to include the related peptones.

Table I. *Heat denaturation of albumin and globulin in milk*

Temp. of heating ° C.	Time of heating min.	Soluble albumin + globulin + proteose N mg. %	Soluble albumin + globulin N mg. %	Percentage of the albumin + globulin denatured
Milk (A)	Unheated	103·5	78·8	—
75	0	91·7	67·0	15·0
	15	36·5	11·8	85·0
	60	28·1	3·4	95·6
	120	27·1	2·4	96·9
80	0	80·1	55·4	29·7
	15	28·6	3·9	95·1
	30	25·3	0·6	99·3
	60	**24·6**	—	100·0
90	15	26·1	1·4	98·2
	30	**24·8**	—	100·0
Milk (B)	Unheated	98·8	76·7	—
80	5	40·5	18·4	76·0
	15	26·3	4·2	94·5
	30	24·3	2·2	97·1
	60	22·6	0·5	99·4
90	0	43·9	21·8	71·6
	5	26·5	4·4	94·3
	15	23·0	0·9	98·8
	30	**22·2**	—	100·0
	60	**22·0**	—	100·0
95	0	33·8	11·7	84·7
	2	29·2	7·1	90·8
	5	22·6	0·5	99·4
	15	**22·2**	—	100·0
	30	22·6	—	(99·4)
	60	23·1	—	(98·8)
Milk (C)	Unheated	105·0	77·2	—
95	5	31·5	3·7	95·2
	30	28·1	0·3	99·6
100	0	31·5	3·7	95·2
	5	29·9	2·1	97·3
	15	**27·8**	—	100·0
	30	28·6	—	(99·0)
Milk (D)	Unheated	93·0	69·9	—
90	0	40·8	17·7	74·7
	5	26·1	3·0	95·7
	15	24·6	1·5	97·9
	30	**23·1**	—	100·0
	60	**23·1**	—	100·0
95	0	33·5	10·4	85·1
	5	24·1	1·0	98·6
	7½	**23·3**	0·2	99·7
	10	**23·3**	0·2	99·7
	15	**23·1**	—	100·0
	30	**23·4**	—	(99·6)
	60	24·1	—	(98·6)
100	0	30·4	7·3	89·6
	2	27·0	3·9	94·4
	4	24·9	1·8	97·4
	10	**23·2**	—	100·0
	30	24·5	—	(98·0)

Discussion of results

(1) *Heat treatment at* 75–100° C.

The method described in (*b*) above precipitates casein and denatured albumin and globulin from the heated samples at a *p*H of approximately 4·7, and the nitrogen contents of the resulting filtrates reveal the progress of denaturation with considerable accuracy. As the non-protein nitrogen contents of samples (A)–(D) were unchanged by the heating, they are not included in Table I; they have been subtracted from the soluble albumin, globulin, proteose and non-protein nitrogen contents—as found by method (*b*)—to provide the figures given in col. 3. These figures show that, in samples (A), (B), (C), and (D), the soluble protein nitrogen contents were quickly reduced to minima of 24·7, 22·1, 27·8, and 23·1 mg. N per cent respectively. These minima, being the proteose nitrogen contents of the unheated samples, have been subtracted from the total soluble protein nitrogen figures of col. 3 to give the true residual soluble albumin and globulin nitrogen figures in col. 4.

The denaturation of albumin and globulin was extremely rapid at temperatures of 75° C. and above, and complete after the milk samples had been heated for about 60 min. at 80° C., 30 min. at 90° C., 10–15 min. at 95° C., and 5–10 min. at 100° C. Continued heating at 95 and 100° C. increased the soluble protein nitrogen above the minima shown in heavy type in col. 3, but the increases were very small, the largest increase observed—sample (D) heated for 30 min. at 100° C.—amounted to only 1·4 mg. N per cent of the milk. The methods used to separate the proteins do not enable one to tell whether this small amount of proteose nitrogen was produced by hydrolysis of denatured albumin and globulin, or by hydrolysis of casein. The increase in proteose nitrogen reduces the apparent percentage of the original albumin and globulin that has been denatured, as shown in brackets in col. 5.

It appears that at temperatures up to 100° C. the initial denaturation of albumin and globulin, which may be extremely rapid, is followed by a very slow hydrolysis which very slightly increases the proteose, but not the non-protein nitrogen contents of the milk samples. These results do not agree with some data on the changes in the nitrogen distribution of heated milk published by Kieferle & Gloetzl(2), for they found increases in the proteose and non-protein nitrogen contents of some samples on heating at as low a temperature as 63° C., but they used less reliable methods for the separation of the various protein fractions.

In the earlier paper(1) dealing with the denaturation of albumin and globulin at temperatures up to 75° C., the total soluble protein nitrogen contents of the raw and heated milk samples were recorded as albumin and globulin nitrogen, and the amounts of protein nitrogen found to be denatured were expressed as a percentage of this total soluble protein nitrogen. The total soluble protein nitrogen has since been shown (pp. 12–13) to contain an

average of 76 per cent of proteose nitrogen, and therefore the percentages of albumin and globulin denatured, as given in that paper, are at about 76/100ths of their real value.

(2) *Heat treatment at* 115 *and* 120° C.

Heating milk samples at 115 and 120° C. resulted in complete denaturation of the albumin and globulin, and appreciable hydrolysis of protein to proteose and to non-protein nitrogenous substances, as shown in Table II.

Table II. *Changes in the proteins of milk heated at* 115 *and* 120° C.

	N fraction, mg. % of milk				N distribution per 100 parts of total N		
Milk sample	Casein + albumin + globulin	Proteose + non-protein	Non-protein	Proteose	Casein + albumin + globulin	Proteose	Non-protein
Unheated	470·7	45·9	24·5	21·4	91·1	4·1	4·8
Heated at 115° C. for 15 min.	466·0	50·7	27·8	22·9	90·2	4·4	5·4
Heated at 115° C. for 30 min.	460·9	55·8	30·0	25·8	89·2	5·0	5·8
Heated at 120° C. for 30 min.	454·7	62·0	32·6	29·4	88·0	5·7	6·3

After heating for 30 min. at 115 and 120° C., the proteose nitrogen contents were 20 and 37 per cent greater than in the raw milk, and the non-protein nitrogen contents 22 and 33 per cent greater. These observations have particular significance with regard to the growth of bacterial cultures in boiled and sterilized milk. It is well known that certain bacteria grow more readily in such heated milks than in raw milk, and this may be due to the increase in simple nitrogenous substances permitting them to satisfy their nitrogen requirements.

SUMMARY

1. The denaturation of albumin and globulin took place rapidly in samples of milk heated at temperatures of 75° C. and above, and was complete in approximately 60, 30, 10–15, and 5–10 min. at 80, 90, 95, and 100° C. respectively.

2. There was no change in the non-protein nitrogen content of milk on heating at temperatures up to 100° C.; on continued heating at 95 and 100° C. extremely small amounts of proteose were produced by hydrolysis of protein.

3. In milk heated at 115 and 120° C. the denaturation of albumin and globulin was followed by appreciable hydrolysis of protein, which resulted in considerable increases in the proteose and in the non-protein nitrogen contents.

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(1) ROWLAND (1933). *J. Dairy Res.* **5**, 46.
(2) KIEFERLE & GLOETZL (1930). *Milchw. Forsch.* **11**, 62.

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147. THE SOLUBLE PROTEIN FRACTION OF MILK

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(With 2 Figures)

The soluble protein fraction of milk is usually considered to be made up entirely of lactalbumin and lactoglobulin, although the view that it may consist partly of secondary proteins of a proteose-peptone nature has been expressed from time to time. If these secondary proteins are present, they are being determined and reported as albumin. An accurate knowledge of the composition of the soluble protein fraction is of importance in the elucidation of the changes which take place when milk is heated, and in the study of the physiology of normal and abnormal milk secretion.

The total amount of soluble protein in normal milk, on the basis of nitrogen content × 6·38, averages 0·56 per cent (1). There are in the literature many data to show that, whereas a soluble protein figure of this order is obtained by the use of protein precipitants, heat coagulation of the casein-free filtrate of milk removes a maximum of about 70 per cent of the soluble protein (2). Such data have pointed to the possibility that some, or all, of the remaining 30 per cent may be present in the original milk as non-coagulable proteose-peptone substances, but have not permitted a definite conclusion for the reason that the heating of albumin and globulin in acid solution would result in incomplete coagulation and the liberation of proteose-like substances by hydrolysis. Palmer & Scott (3), and Porcher (4) discussed the possibility of the presence of secondary proteins in fresh milk, and dismissed it owing to lack of proof. Osborne & Wakeman (5) stressed the importance of the problem and stated that, although their own experiments gave no positive conclusions, no method they had devised enabled them to say definitely that proteose was not an original constituent of milk. Kieferle & Gloetzl (6), and Kieferle (7) have recorded the soluble proteins which are not coagulable by heat, but precipitable by phosphotungstic acid, as proteose and peptone, and Jones & Little (8) claimed to have shown the presence of considerable amounts of proteose in milk by precipitating the casein-free filtrate with two concentrations of trichloracetic acid.

The writer's attention was directed to the proteose problem during an investigation of the extent to which denaturation of albumin and globulin and degradation of protein take place when milk is heated at high temperatures. The results that were obtained can be interpreted only on the assumption that secondary proteins are present in milk. It was realized that a definite conclusion would be possible if the conditions necessary for the quantitative

coagulation of albumin and globulin could be established, or a reliable precipitation method for the separation of these proteins from secondary proteins worked out; and an attempt was made to get information by both means.

(1) *Heat coagulation of albumin and globulin.* Much more work has been done on the optimum conditions for the heat coagulation of the soluble proteins of egg and blood than of milk. The extensive studies carried out by Sørensen and his associates have emphasized that the maximum precipitation of egg albumin was obtained by adjusting its solution to the isoelectric point and boiling for a short time, and that, even on prolonged boiling at the isoelectric point, only an extremely small proportion of the original albumin was hydrolysed (9). Hendrix & Wilson (10) found pure recrystallized dialysed egg albumin to be completely precipitated by heating its solution at its isoelectric point, and Hewitt (11) observed complete precipitation of horse serum albumin on boiling at *p*H 4·75; on either side of this *p*H the extent of precipitation fell off rapidly. Sørensen (12) and Michaelis & Nakashima (13) reported the isoelectric point of egg albumin to be *p*H 4·8 and 4·75 respectively.

Applying these observations to the quantitative coagulation of the albumin and globulin of milk, it was clear that satisfactory results would be obtained only by the most careful control of the *p*H of the casein-free filtrate. The isoelectric point of lactalbumin was given as *p*H 4·55 by Csonka *et al.* (14), and 5·2 by Sjogren & Svedberg (15). No figures were found for lactoglobulin; for the serum globulin with which it is probably identical the isoelectric point of *p*H 5·4 has been determined by electrophoresis (16, 17, 18). Okuda & Zoller (19) found maximum precipitation of the mixed proteins of whey to occur at *p*H 4·5. It was decided to ascertain experimentally the *p*H at which the maximum coagulation of the soluble proteins of milk could be effected.

(2) *The choice of a protein precipitant.* The only precipitant which appeared promising for the separation of whole protein from proteose was trichloracetic acid. It has been accepted by a number of workers on blood and protein digests as a reagent giving a sharp separation. Sjollema & Hellerschy (20), Hahn (21), and Hiller & Van Slyke (22) found that low concentrations (2–5 per cent), used at room temperature, precipitated whole protein only, and that high concentrations precipitated secondary proteins in addition. Wasteneys & Borsook (23), in their method for the "analysis of incomplete hydrolysates", used 2 per cent trichloracetic acid for the precipitation of whole proteins. Jones & Little (8) considered the precipitate obtained from the casein-free filtrate of milk with 5 per cent trichloracetic acid to consist of whole proteins, and with 10 per cent whole proteins plus proteose.

As the concentrations of trichloracetic acid used for the precipitation of whole protein from blood and protein digests might not be directly applicable to the casein-free filtrate of milk, this filtrate has been precipitated with solutions of trichloracetic acid covering a wide range of concentrations.

Experimental I

(1) *The effect of pH on the heat coagulation of albumin and globulin*

The casein was precipitated from a sample of milk by a modification of Moir's method (24); 1 ml. of 10 per cent acetic acid solution and 1 ml. of 1*N* sodium acetate solution were added per 10 ml. of milk, and the mixture diluted finally to 50 ml. From a bulk of casein-free filtrate so prepared, 50 ml. portions were adjusted to different *p*H's by the addition of either sodium hydroxide or sulphuric acid solutions, boiled for 10 min., cooled, made up to the original volume, and filtered through a dry paper. The nitrogen content of these filtrates, and of the unheated casein-free filtrate, was determined by the Kjeldahl method, and the amounts of albumin and globulin nitrogen coagulated were calculated. The results of the first preliminary experiment are recorded in Table I (*a*), and of a more comprehensive experiment on a second sample of milk in Table I (*b*). These results show maximum coagulation at *p*H 4·75–4·80. The measurements of *p*H were made with the quinhydrone electrode.

Table I. *Effect of pH on the heat coagulation of albumin and globulin*

Exp.	*p*H of casein-free filtrate	mg. protein N coagulated per 100 ml. of milk
(*a*)	4·64	71·4
	4·92	72·4
	5·23	68·3
	5·81	63·5
	7·23	55·4
(*b*)	4·00	50·9
	4·35	61·2
	4·66	67·8
	4·83	68·1
	5·05	65·9
	5·32	62·7
	5·67	57·1
	6·01	52·5
	6·57	47·8

The effect of prolonged heating at the *p*H of maximum coagulation was investigated by keeping portions of a casein-free filtrate (*p*H 4·75) immersed in boiling water for periods ranging from 5 to 60 min. No difference was detected in the amounts of protein coagulated, the filtrates after removal of coagulated protein were all perfectly clear, and gave no opalescence on further heating.

(2) *Trichloracetic acid as a precipitant of albumin and globulin*

To 30 ml. portions of a casein-free filtrate obtained as in (1) above, varying amounts of 50 per cent trichloracetic acid solution and water were added to give a constant volume of 50 ml. The milk-acid mixtures were filtered through dry papers, and the nitrogen content of these acid filtrates, and of the untreated casein-free filtrate, was determined by the Kjeldahl method. The

amounts of protein nitrogen precipitated were calculated, and are shown in Table II, for concentrations of trichloracetic acid from 1 to 20 per cent; (*a*) being a preliminary series, and (*b*) a carefully planned final series on another milk sample.

The amounts of protein nitrogen coagulated by heating, at *p*H 4·75, the casein-free filtrates from the two milk samples are also included for comparison in Table II.

Table II. *The precipitation of the soluble proteins of milk by trichloracetic acid*

Exp.	Concentration of trichloracetic acid (%)	mg. protein N precipitated per 100 ml. of milk
(*a*)	2·0	49·0
	4·0	60·4
	6·0	94·8
	8·0	95·8
	10·0	96·0
	12·0	95·8
	16·0	95·3
	20·0	95·3
Heat coagulation at *p*H 4·75		68·1
(*b*)	1·0	37·0
	2·0	47·4
	3·0	55·2
	4·0	65·7
	4·4	74·0
	4·7	83·3
	5·0	92·7
	6·0	97·9
	8·0	98·3
	10·0	98·3
	12·0	98·0
Heat coagulation at *p*H 4·75		74·0

Discussion of results

(1) *Heat coagulation of albumin and globulin.*

The data of Table I (*b*), represented graphically in Fig. 1, definitely indicate that on heating the casein-free filtrate of milk, the maximum coagulation of albumin and globulin takes place at *p*H 4·75–4·80. It is obvious that for the removal of these proteins by heat coagulation the *p*H of the solution must be controlled within very narrow limits.

(2) *Trichloracetic acid precipitation of the casein-free filtrate.*

The data of Table II (*b*), represented graphically in Fig. 2, show increasing precipitation of proteins with increasing concentration of trichloracetic acid, up to a maximum of 8–10 per cent, but concentrations of acid much above this, even up to 20 per cent, give very nearly as large a precipitate, showing that the hydrolytic action of the acid on proteins is extremely slow at room temperature.

The most interesting and important part of the curve is the range from 4·5 to 5·5 per cent acid, for small increases in the concentration of acid cause relatively large increases in the amount of protein precipitated, and might well correspond with the precipitation of a more or less homogeneous protein

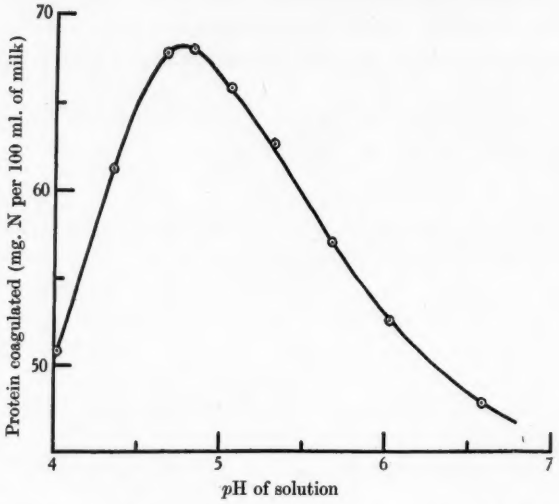

Fig. 1. Effect of *p*H on the heat coagulation of albumin and globulin.

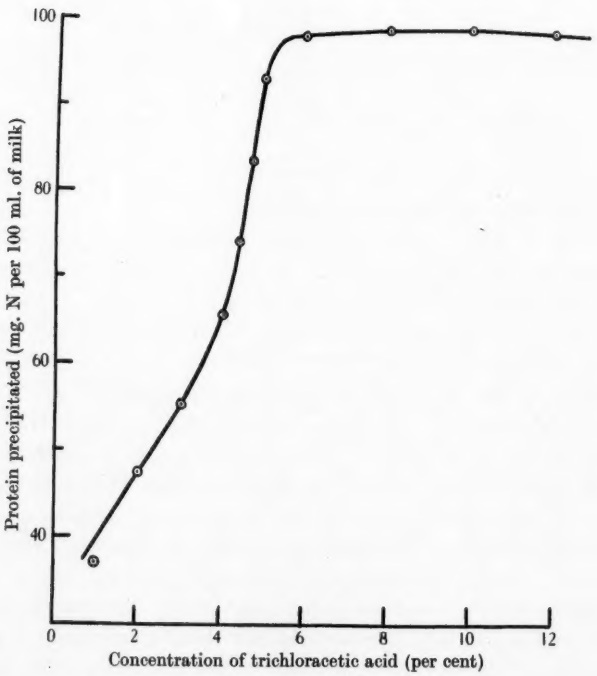

Fig. 2. The precipitation of the soluble proteins of milk by trichloracetic acid.

fraction. In this connexion it will be seen that, with milk samples (*a*) and (*b*), heat coagulation at *p*H 4·75 gave the same amount of precipitate as 4·4 per cent trichloracetic acid. As high concentrations of the acid are known to precipitate secondary proteins, it is reasonable to suggest that the protein substances precipitated by 10 per cent acid, and not precipitated by 4·4 per cent, are of a proteose-peptone nature.

The data presented also show that concentrations of the acid as low as 2–2·5 per cent, although employed for the precipitation of whole proteins from blood and protein digests, are not satisfactory when used with the casein-free filtrate of milk, as they precipitate less protein than heat coagulation.

EXPERIMENTAL II

It is possible to effect heat coagulation of the albumin and globulin of milk by two methods; (1) by boiling the casein-free filtrate at *p*H 4·75, when the denaturation of the proteins is followed immediately by their flocculation, and (2) by heating the milk itself, and later, co-precipitating the denatured proteins along with casein at *p*H 4·7. Both methods have been applied to a number of milk samples. It was found that an average of about 70 per cent of the total soluble protein nitrogen of the samples was coagulated by method (1) but that an average of about 76 per cent was the maximum coagulated by method (2). The results obtained with one of the samples will be given in detail and used as an example for discussion.

On the raw milk, and on the portions of it that had been heated at various temperatures, the following determinations of nitrogen were made by a semi-micro Kjeldahl method; (*a*) total N, (*b*) non-protein N by trichloracetic acid precipitation of the total protein substances, (*c*) N in the filtrate after removal of casein, and denatured albumin and globulin, at *p*H 4·7, and (*d*) N coagulated by boiling that filtrate at *p*H 4·75. For details of the methods of analysis and heat treatment the reader is referred to p. 1.

Results

The composition of the raw milk is shown in Table III, and the essential figures concerning the heated portions of this sample in Table IV. There was no change in the total or non-protein N contents as a result of the heat treatment. The figures in col. 3 of Table IV are the differences (*c*) minus (*b*).

Table III. *Composition of the raw milk*

N fraction	mg. %
Total N	532·7
Casein N	410·5
Non-protein N	29·2
Total soluble protein N	93·0
Albumin and globulin N (heat coagulation of casein-free filtrate at *p*H 4·75) ...	64·1

Table IV. *Denaturation of albumin and globulin on heating the milk*

Temp. of heating ° C.	Time of heating min.	Residual soluble protein N mg. % of milk	Albumin and globulin N denatured mg. % of milk	Percentage of total soluble proteins denatured
90	0	40·8	52·2	56·1
	5	26·1	66·9	72·0
	15	24·6	68·4	73·6
	30	23·1	69·9	**75·2**
	60	23·1	69·9	**75·2**
95	0	33·5	59·5	63·9
	5	24·1	68·9	74·1
	7½	23·3	69·7	**74·9**
	10	23·3	69·7	**74·9**
	15	23·1	69·9	**75·2**
	30	23·4	69·6	**74·8**
	60	24·1	68·9	74·1
100	0	30·4	62·6	67·3
	2	27·0	66·0	71·0
	4	24·9	68·1	73·2
	10	23·2	69·8	**75·1**
	30	24·5	68·5	73·7

Discussion of results

The maximum amount of albumin and globulin N coagulated by boiling the casein-free filtrate was 64·1 mg. per cent, and the maximum amount precipitated in the denatured form along with casein 69·9 mg. per cent of the milk. These amounts represent 68·9 and 75·2 per cent of the total soluble protein N.

The possibility that the greater removal of albumin and globulin by the second method was due to the adsorption of small amounts of non-coagulable secondary protein by the precipitate of casein and denatured albumin and globulin, was disposed of by the figures for the amounts of protein coagulated by boiling the filtrates from the heated milks. In the case of the samples heated to 90 and 95° C. and cooled immediately, it will be seen that 56·1 and 63·9 per cent respectively of the total soluble protein N were coagulated with the casein. Boiling the filtrates at *p*H 4·75 coagulated more protein and raised the total in each case to 68·9 per cent, which was the amount removed by boiling the casein-free filtrate from the unheated milk. Boiling the filtrates of the other heated milk samples, from which more than 68·9 per cent, and less than 75·2 per cent of the total soluble protein N had already been removed along with casein, produced a very faint opalescence but no filterable coagulable coagulum. The difficulty of coagulating soluble proteins completely by heating their solutions has been emphasized earlier. The increased removal of albumin and globulin that has been attained by their precipitation in the denatured form along with casein is in accord with the well-known phenomenon of sympathetic coagulation of colloids.

Boiling the casein-free filtrate of the raw milk at *p*H 4·75 left albumin and globulin N amounting to 5·8 mg. per cent of the milk in solution. For the casein precipitation 10 ml. of the milk were diluted to 100 ml., therefore the

albumin and globulin N not removed amounted to only 0·58 mg. per 100 ml. of solution—an extremely low concentration. The co-precipitation of casein and denatured albumin and globulin facilitated the precipitation of the last traces of the denatured protein.

The increased removal of albumin and globulin N discussed above represented 1·1 per cent of the total N of the milk. Similar experiments with other milk samples gave figures ranging from 0·95 to 1·1.

The above remarks have been based on the assumption that the maximum coagulation of albumin and globulin which followed the heating of the milk samples at 90° C. for 30 min., 95° C. for 15 min., and 100° C. for 10 min. was complete coagulation, and that the total soluble protein N consisted of 75·2 per cent albumin and globulin N and 24·8 per cent N in the form of non-coagulable secondary proteins of a proteose-peptone nature. It is difficult to see how any other conclusion could be reached from the data of Table IV. The results obtained at 95° C., for example, show that after 7½ min. there was no further denaturation of soluble protein, and it was not until after 30 min. that an increase of soluble protein by hydrolysis commenced. Even after 60 min. this increase was almost negligible, being from 23·4 to 24·1 mg. N per cent. Such a slow rate of hydrolysis is inadequate to account for the failure to denature 24·8 per cent of the total soluble protein N, while the very rapid rate at which denaturation takes place (63·9 and 74·1 per cent of the total soluble protein N denatured in 0 and 5 min. respectively) makes it highly improbable that the 74·8–75·2 per cent of the total soluble protein N denatured in 7½–30 min. does not coincide with complete denaturation.

The data that have been given for one milk sample have been confirmed by similar data for several other samples, and lead to the conclusion that proteose-peptone substances are present in fresh milk.

The writer has determined the total, albumin and globulin, and proteose-peptone N contents of fifteen samples of normal milk. The albumin and globulin N was found to constitute 12·5, and the proteose-peptone N 4·0 per cent of the total N, as an average; corresponding with a total soluble protein N fraction composed of 75·8 per cent albumin and globulin N, and 24·2 per cent proteose-peptone N.

SUMMARY

1. The literature on the soluble protein fraction of milk has been reviewed to show the difficulties that are encountered in attempting to decide whether the fraction consists, as is usually supposed, of lactalbumin and lactoglobulin only, or whether it consists partly of secondary proteins of a proteose-peptone nature.

2. It has been shown that on boiling the casein-free filtrate of milk, maximum coagulation of albumin and globulin occurs at a *p*H of 4·75–4·80. At this *p*H prolonged boiling does not cause hydrolysis, and the amount of

albumin and globulin N coagulated represents about 70 per cent of the total soluble protein N of milk.

3. By precipitation of the casein-free filtrate of milk with various concentrations of trichloracetic acid, a partition of the soluble proteins has been effected which strongly suggests the presence of proteose-peptone substances to an extent in agreement with the results of heat coagulation.

4. The maximum amount of albumin and globulin rendered coagulable by heating milk itself has been determined by precipitating denatured albumin and globulin along with casein at *p*H 4·7. This maximum represented an average of 76 per cent of the total soluble protein N in a series of normal milk samples, and reasons have been given for considering this maximum as the total of true albumin and globulin present.

5. The conclusion has been reached that the soluble protein fraction of normal fresh milk is composed of approximately 76 per cent albumin and globulin, and 24 per cent proteose-peptone substances.

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148. DIACETYL IN COLD-STORED BUTTERS. II

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(With 1 Figure)

The market value of well-manufactured butter is determined more by its flavour than by any other property, and the problem of the identification and estimation of the flavouring substances is therefore of considerable importance.

Schmalfuss (9) and Van Niel *et al.* (11) were the first to show that the aroma of butter made from ripened cream is related to its diacetyl content, and a considerable amount of work has since been done, notably by Hammer and his associates (4), on the development of diacetyl in starter cultures.

Previous work by the author (2) indicated that (*a*) "total diacetyl"[1] in New Zealand starter butters is mainly added with the starter, rather than developed by the customary mild ripening process, (*b*) the "total diacetyl" in butter appears to be retained with the butter-milk fraction, (*c*) acetylmethylcarbinol is lost to only a slight extent during 6 months' cold storage at 14° F., and (*d*) there is a gradual loss of diacetyl during the period of storage.

The experiments reported in this paper were made in order to study the derivation of diacetyl in starter butters, and to follow the distribution of diacetyl and its precursor acetylmethylcarbinol[2] during the butter-making process. The changes taking place during the period of cold storage were also observed.

Experimental

The following aspects of the problem were studied:

(1) The effect of neutralization.

(2) The distribution of diacetyl and carbinol during the manufacture of butter.

(3) The retention of diacetyl during storage.

(4) The relation of diacetyl to the aroma and keeping quality of butter.

(5) The influence of diacetyl on the flavour and grade of "mild starter" butter.

(6) and (7) The development of diacetyl in starter cultures and in butter.

Manufacture

The butters were made from finest cream pasteurized at approximately 200° F. and churned at a temperature of 40–50° F. The butters contained 15–16 per cent of moisture, 1·0–1·6 per cent of salt and 0·5 per cent of curd. Other details of manufacture are given in Table I.

[1] "Total diacetyl" = diacetyl and acetylmethylcarbinol, which are estimated together.

[2] Hereinafter referred to as "carbinol".

Table I

Butter No.	Date of manufacture	Cream: Raw cream acidity %	Cream: Neutralizer added	Cream: Acidity on delivery to vat	Starter: Acidity %	Starter: Added %	Ripening process. Acidity of cream: After addition of starter	Ripening process. Acidity of cream: When churned	Butter. *p*H value when freshly made
1*a*	7. v. 35	0·09	Nil	0·06	0·97	1·0	0·07	0·18	—
b	,,	0·09	Nil	0·06	0·94	16·2	0·18	0·18	—
2*a*	13. v. 35	0·07	Nil	0·055	—	0·5	0·055	0·07	—
b	,,	0·18*	Sod. bic.	0·06	—	0·5	0·06	0·075	—
3	3. xii. 35	0·115	Nil	0·08	0·85	10·0	0·16	0·35	5·55
4*a*	7. i. 36	0·11	Nil	0·08	—	Nil	—	0·08	6·6
b	,,	0·11	Nil	0·08	—	Nil	—	0·08	6·6
c	,,	0·11	Nil	0·08	—	Nil	—	0·08	6·7
5*a*	14. i. 36	0·10	Nil	0·075	—	Nil	—	0·075	6·6
b	,,	0·10	Nil	0·075	—	Nil	—	0·075	6·6
c	,,	0·10	Nil	0·075	—	Nil	—	0·075	6·6
6*a*	21. i. 36	0·10	Nil	0·08	0·91	1·4	0·095	0·12	6·6
b	,,	0·10	Nil	0·08	0·91	1·4	0·095	0·13	6·6
c	,,	0·10	Nil	0·08	0·91	1·4	0·095	0·14	6·4
7*a*	25. ii. 36	0·10	Nil	0·085	0·95	1·0	0·10	0·12	6·6
b	,,	0·10	Nil	0·085	0·95	1·0	0·10	0·115	6·6
c	,,	0·10	Nil	0·085	0·95	1·0	0·10	0·12	6·4
8*a*	3. iii. 36	0·105	Nil	0·085	—	Nil	—	0·085	6·6
b	,,	0·105	Nil	0·085	—	Nil	—	0·085	6·6
c	,,	0·105	Nil	0·085	—	Nil	—	0·085	6·6
9*a*	10. iii. 36	0·11	Nil	0·08	—	Nil	—	0·08	6·6
b	,,	0·11	Nil	0·08	—	Nil	—	0·08	6·6
c	,,	0·11	Nil	0·08	—	Nil	—	0·08	6·6
10*a*	26. iii. 36	0·30*	Sod. bic.	0·115	—	Nil	—	0·12	—
b	,,	0·30*	Sod. bic.	0·08	—	Nil	—	0·085	—
c	,,	0·30*	Sod. bic.	0·07	0·86	4·0	0·105	0·135	—
d	,,	0·30*	Sod. bic.	0·07	0·76	8·3	0·135	0·135	—
11*a*	31. iii. 36	0·10	Nil	0·10	—	Nil	—	0·10	6·6
b	,,	0·10	Nil	0·10	0·92	2·0	0·12	0·13	6·5
c	,,	0·10	Nil	0·10	0·92	6·0	0·155	0·18	6·4
12*a*	2. iv. 36	0·30*	Sod. bic.	0·13	—	Nil	—	0·145	6·4
b	,,	0·30*	Sod. bic.	0·05	—	Nil	—	0·065	7·2
c	,,	0·30*	Sod. bic.	0·055	0·92	5·0	0·10	0·145	6·4
d	,,	0·30*	Sod. bic.	0·055	0·86	9·6	0·145	0·145	6·5

* Finest cream ripened with starter to simulate natural souring.

Estimation of diacetyl and carbinol

The method was that already reported by the author (1). Every effort was made to keep the cream and butter-milk unchanged pending analysis, by maintaining them at low temperatures and by adding sodium chloride. The butters were held at approximately 40° F. for 1 day before examination.

In Table II, the diacetyl contents of the cream and butter-milk during the course of manufacture are given, and the changes in the amounts of these constituents in butter stored (*a*) for several days at chilling temperature (40° F.) and (*b*) for 3½ months or longer at freezing temperatures (14° F.) are recorded.

In Table IIA the distribution of the diacetyl and carbinol between the cream and the butter, calculated from the results in Table II, is recorded.

Table II

Butter No.	Churning Date	Cream: Initial D.	Cream: Initial C.	Cream: Starter D.	Cream: Starter C.	Cream: Starter Added %	Cream: Added as synthetic compound D.	Cream: Added as synthetic compound C.	Cream: At churning D.	Cream: At churning C.	Buttermilk D.	Buttermilk C.	Wash water "Total D."	Butter: 1 day after manufacture D.	Butter: 1 day after manufacture C.	Butter: 7–10 days after manufacture at 40° F. D.	Butter: 7–10 days after manufacture at 40° F. C.	Butter: After frozen storage for 3½–7½ months D.	Butter: After frozen storage for 3½–7½ months C.
1*a*	7. v. 35	—	0·1	60·5		1·0	Nil	Nil	10·0		20·1		—	0·2	1·5	0·2	1·6	Nil	1·25
b	,,	—	0·1	68·5		16·2	Nil	Nil	11·1		20·1		0·8	0·15	1·5	0·15	1·5	Trace	1·3
2*a*	13. v. 35	—	0·1	82		0·5	Nil	Nil	1·2		22·6		—	Trace	0·2	0·1	—	Nil	0·15
b	,,	9·5		82		0·5	Nil	Nil	9·2		22·6		0·8	0·05	1·35	0·2	—	Nil	0·7
3	3. xii. 35	—	—	—	—	—	Nil	Nil	—	—	—	—	—	—	—	1·1	11·2	1·5	9·4
4*a*	7. i. 36	—	—	Nil	Nil	Nil	Nil	Nil	—	0·2	0·45		—	Nil	0·2	Nil	0·4	0·1	0·15
b	,,	—	—	Nil	Nil	Nil	0·3	7·4	—	—	7·6		—	Nil	2·1	0·25	2·1	0·2	1·8
c	,,	—	—	Nil	Nil	Nil	0·3	7·4	4·3		8·5		—	0·05	2·0	0·25	1·95	0·1	2·15
5*a*	14. i. 36	1·1		Nil	Nil	Nil	Nil	Nil	0·1	1·0	1·7		—	0·1	0·4	Nil	0·3	0·1	0·3
b	,,	1·1		Nil	Nil	Nil	0·4	7·2	0·45	6·35	9·6		—	0·25	1·75	0·1	2·1	0·15	1·95
c	,,	1·1		Nil	Nil	Nil	0·4	7·2	0·35	6·05	9·0		—	0·2	2·1	0·2	1·8	0·2	1·9
6*a*	21. i. 36	—	—	—	—	1·4	Nil	Nil	0·5	6·1	11·4		—	0·25	1·55	0·30	2·0	0·2	1·6
b	,,	—	—	—	—	1·4	0·4	7·2	0·8	14·2	23·1		—	0·4	3·3	0·65	3·35	0·35	3·25
c	,,	—	—	—	—	1·4	0·4	7·2	1·0	17·0	31·5		—	0·4	4·9	0·95	4·05	0·5	4·5
7*a*	25. ii. 36	—	0·3	138		1·0	Nil	Nil	0·35	2·25	5·9		—	0·1	1·1	0·1	1·1	0·3	1·0
b	,,	—	0·3	138		1·0	0·4	7·2	7·8		12·7		—	0·35	2·55	0·35	2·45	0·3	2·8
c	,,	—	0·3	138		1·0	0·4	7·2	8·8		14·0		—	0·25	2·45	0·25	1·95	0·4	1·7
8*a*	3. iii. 36	—	—	Nil	Nil	Nil	Nil	Nil	Nil	0·3	0·1	—	—	0·1	—	Nil	—	Nil	—
b	,,	—	—	Nil	Nil	Nil	1·0	Nil	0·8	—	1·2	—	0·15*	0·3	—	0·1	—	0·2	—
c	,,	—	—	Nil	Nil	Nil	2·0	Nil	1·9	—	2·2	—	0·45*	0·45	—	0·25	—	Nil	—
9*a*	10. iii. 36	—	—	Nil	Nil	Nil	Nil	Nil	0·1	0·2	Nil	—	—	Nil	—	Trace	—	0·1	—
b	,,	—	—	Nil	Nil	Nil	4·0	Nil	3·2	—	4·2	—	0·5*	0·8	—	0·8	—	0·8	—
c	,,	—	—	Nil	Nil	Nil	6·0	Nil	5·0	—	7·7	—	0·3*	1·1	—	1·2	—	1·0	—
10*a*	26. iii. 36	0·65	14·0	Nil	Nil	Nil	Nil	Nil	0·4	14·3	—	—	—	0·3	2·4	0·1	2·5	0·2	1·9
b	,,	0·5	10·6	Nil	Nil	Nil	Nil	Nil	0·8	14·8	—	—	—	0·3	2·1	Nil	2·3	0·15	2·15
c	,,	0·5	10·6	5·0	104	4	Nil	Nil	0·95	19·1	—	—	—	0·3	3·3	0·3	3·3	0·3	2·9
d	,,	0·5	10·6	5·5	95	8·3	Nil	Nil	0·95	15·35	—	—	—	0·45	3·05	0·2	3·3	0·3	3·4
11*a*	31. iii. 36	—	—	Nil	Nil	Nil	Nil	Nil	—	—	—	—	—	Nil	0·35	Nil	—	Nil	0·1
b	,,	—	—	—	—	2·0	Nil	Nil	—	—	—	—	—	0·2	1·5	0·15	—	0·2	1·8
c	,,	—	—	—	—	6·0	Nil	Nil	—	—	—	—	—	0·6	4·2	0·5	—	0·5	4·9
12*a*	2. iv. 36	—	9·4	Nil	Nil	Nil	Nil	Nil	0·55	8·65	—	—	—	0·1	1·6	Nil	—	0·2	1·5
b	,,	0·45	7·4	Nil	Nil	Nil	Nil	Nil	0·45	6·75	—	—	—	Nil	1·6	0·1	—	Nil	1·5
c	,,	0·45	7·4	3·9	126	5·0	Nil	Nil	1·25	15·05	—	—	—	0·35	3·75	0·2	—	0·45	3·25
d	,,	0·45	7·4	4·1	104	9·6	Nil	Nil	1·3	11·0	—	—	—	0·25	3·15	0·15	—	0·2	3·0

* Churn not drained.

D. = diacetyl; C. = carbinol.

Table II A

Butter No.	Churning Date	Distribution of diacetyl and carbinol during manufacture: Fat % in cream at churning	% retained in butter: Diacetyl	% retained in butter: Carbinol
1*a*	7. v. 35	—	—	—
b	,,	—	—	—
2*a*	13. v. 35	—	—	—
b	,,	—	—	—
3	3. xii. 35	—	—	—
4*a*	7. i. 36	—	—	—
b	,,	—	—	—
c	,,	36·5	—	20
5*a*	14. i. 36	37·0	—	18
b	,,	37·0	—	12
c	,,	37·0	—	12
6*a*	21. i. 36	38·5	23	12
b	,,	38·5	23	11
c	,,	38·5	18	18
7*a*	25. ii. 36	37·0	—	22
b	,,	—	—	—
c	,,	—	—	—
8*a*	3. iii. 36	—	—	—
b	,,	41·0	18	—
c	,,	41·0	12	—
9*a*	10. iii. 36	—	—	—
b	,,	40·0	12	—
c	,,	40·0	11	—
10*a*	26. iii. 36	36·0	33	7
b	,,	36·0	16	6
c	,,	34·5	13	7
d	,,	33·0	18	8
11*a*	31. iii. 36	—	—	—
b	,,	—	—	—
c	,,	—	—	—
12*a*	2. iv. 36	35·0	8	8
b	,,	35·0	Nil	10
c	,,	33·5	11	10
d	,,	31·5	7	11

For this calculation it has been assumed that the "over-run" of the butter was 20 per cent. The following is an example of the method of calculation (Butter 5*b*):

Percentage of fat in cream when churned = 37; therefore 100 lb. of fat yield 44·5 lb. of butter.

100 parts of cream containing 6·35 p.p.m. carbinol = 635

44·5 ,, butter ,, 1·75 ,, = 79

$$\text{Carbinol retained by butter} = \frac{79 \times 100}{635} = 12 \text{ per cent.}$$

DISCUSSION

(i) *The effect of neutralization and pasteurization on the diacetyl and carbinol in cream*

In the absence of any other criterion, it was considered that the loss of the diacetyl and carbinol might be regarded as an indication of the effect of neutralization and pasteurization on the flavouring substances of cream. The results are recorded in Table III. (Low acid creams containing smaller

Table III. *Diacetyl and carbinol contents in p.p.m.*

Pasteurizer ...	A				B			
	Diacetyl		Carbinol		Diacetyl		Carbinol	
	Content	Loss %	Content	Loss %	Content	Loss %	Content	Loss %
(1) *Initial*, cream ripened to 0·30% acidity	0·55	Nil	16·3	Nil	0·50	Nil	10·6	Nil
(2) Cream neutralized to (*a*) 0·12% and (*b*) 0·13% acidity	0·66	Gain	14·0	14	0·53	Gain	8·7	17
(3) Cream over-neutralized to (*a*) 0·075% and (*b*) 0·050% acidity	0·50	10	10·6	35	0·45	10	6·8	36

amounts of carbinol (2 p.p.m. and less) lose only a portion of this substance during pasteurization.) It is therefore apparent that the processes of neutralization and pasteurization (*a*) cause no notable decrease in the content of the aroma substance diacetyl, (*b*) produce a distinct loss of the precursor substance carbinol, which is parallel with the neutralization point of the cream, and (*c*) give similar results with the two types of pasteurizer examined.

Although the carbinol is flavourless, it contains both a carbonyl and a hydroxyl group which are typical of many natural flavouring substances. The destruction of the carbinol by the neutralizer may therefore have some bearing on the opinion, frequently expressed, that butters made from over-neutralized cream are deficient in flavour.

(ii) *The distribution of diacetyl and carbinol during the course of manufacture*

Diacetyl. The percentage of diacetyl retained by the butter varied from 0 to 33 per cent, the extremes being found with neutralized creams. The average retention in the butter was 15 per cent of the amount originally present in the cream (Tables II and IIA).

Acetylmethylcarbinol. When carbinol was added as the synthetic compound[1]

[1] E. K. Co. Technical product containing 3–4 per cent of diacetyl and by hydroxylamine titration, after allowing for the diacetyl, approximately 73 per cent of ketone (calculated as acetylmethylcarbinol).

to pasteurized cream there was, in the absence of starter, a loss before churning (series 4 and 5). Results obtained for twelve churnings show that from 7 to 22 (averaging 14) per cent of the carbinol present in the cream was retained by the butter (series 4*c*, 5, 6, 7*a*, 10*c*, *d* and 12*c*, *d*).

Neither the diacetyl nor the carbinol is concentrated in the fat during manufacture, as believed by some workers(3, 5). Approximately one-sixth (17 per cent) of the butter-milk in cream is retained in New Zealand butter(10), and the foregoing results indicate that carbinol and diacetyl are distributed with the butter-milk throughout the process of manufacture.

For practical purposes, it may be assumed that the ratio of diacetyl in butter to diacetyl in cream (35–40 per cent fat) is 0·4.

Table IV. *Ratio of diacetyl* $\frac{butter}{cream}$ *(starter butters)*

Churning No.	Diacetyl results in p.p.m.		Ratio diacetyl butter/cream
	Cream	Butter	
6*a*	0·5	0·25	0·5
b	0·8	0·4	0·5
c	1·0	0·4	0·4
7*a*	0·35	0·1	0·3
10*c*	0·95	0·3	0·3
12*c*	1·25	0·35	0·3
Average	0·8	0·3	0·4

The removal of "total diacetyl" by the washing process. Great emphasis is laid by some writers on the possibility of the removal of flavouring substances by washing. The loss of "total diacetyl" found under local manufacturing conditions, however, is relatively unimportant, and it would appear that these substances are mainly removed with butter-milk remaining in the churn after draining.

(iii) *The retention of diacetyl and carbinol by butter during storage*

A portion of each experimental butter was kept for 7–10 days at 40° F. The main sample was frozen for approximately 3½ months and was allowed to thaw for about 1 week before examination.

In Table II it will be seen that the diacetyl contents changed only slightly during storage. The results are summarized in Table V.

It will be seen that "mild starter" butters contained about eight times the amount of diacetyl found in sweet cream butters, but only one-fourth of the amount present in highly flavoured butters made in accordance with European and American practice. During relatively short periods of storage (3½ months) at 14° F. the diacetyl remains fairly constant, when added as the artificial substance (i) a decrease was observed, but the starter butters ((iv) and (v)) tended to increase slightly.

Table V. *The diacetyl content of butter during storage*

Type of butter	Results in p.p.m. 1 day after manufacture (40° F.)	7–10 days after manufacture (40° F.)	3½ months at 14° F. and approx. 1 week at 40–50° F.
Non-starter:			
(i) Diacetyl added (8*b*, *c*, 9*b*, *c*)	0·66	0·59	0·50
(ii) Diacetyl present with carbinol added (4*b*, *c*, 5*b*, *c*); neutralized creams (10*a*, *b*, 12*a*, *b*)	0·30	0·25	0·30
Starter:			
(iii) Cream ripened (6*a*, 7*a*, 11*b*, *c*)	0·29	0·26	0·30
(iv) Cream containing carbinol (6*b*, *c*, 7*b*, *c*) or products of natural ripening (10*c*, 12*c*)	0·34	0·45	0·38
(v) High acid butter: 0·30% acidity in cream (3)	—	1·1	1·5
(vi) Sweet cream: no starter added (4*a*, 5*a*, 8*a*, 9*a*, 11*a*)	0·04	Nil	0·06

(iv) *The relation of diacetyl to the aroma and keeping quality of butter*

Grading results for the butters recorded in Tables I and II are given in Table VI.

From the results given in Table VI, it will be seen that there is no connexion between the diacetyl content of butter and the grading points allotted. The carbinol content also varied widely without the flavour being commented upon. In New Zealand grading practice, a "full" flavour is not desired, because it is usually associated with a high acidity, which is detrimental to the keeping quality of the butter. As butters with a pronounced "ripened cream" flavour are penalized, the lack of correlation between the grading and the diacetyl content is not unexpected.

The starter butters which have proved to be quite acceptable to the graders contained, as a rule, 0·2–0·4 p.p.m. diacetyl and 1–3 p.p.m. of carbinol. In the absence of starter, as much as 0·8 p.p.m. diacetyl has been present (9*b*) without giving rise to any adverse comments concerning the "fullness" of flavour.[1] This fact, in conjunction with findings recorded elsewhere in this paper, indicates that diacetyl is not the only important aroma substance in starter butter, and it probably contributes only about one-third of the flavour in butter of the "mild starter" type. It is probable, however, that a trace of diacetyl is of considerable importance in imparting "bloom" to butters made from mildly ripened cream.

Results of considerable practical interest have been obtained with butters made from highly acid creams. Butter made commercially from highly acid creams after neutralization is usually affected by off-flavours caused by organisms of a putrefactive type. When the souring has been developed by starter organisms and is of the "clean starter" type, the butter made from

[1] Providing the butter is not too new. In fresh butter, added diacetyl is apparently unevenly distributed and the flavour is rather sickly.

Table VI

Butter No.	Churning date	Butter—initial: Grading points for flavour	Butter—initial: Remarks	Storage conditions: Temp. °F.	Storage conditions: Months	Butter—final: Grading points for flavour	Butter—final: Remarks	Loss in grading points during storage
1*a*	7. v. 35	—	Very full	14–17	7½	39	Stale	—
b	„	—	Milder than (1*a*)	14–17	7½	41½	Rather stale	—
2*a*	13. v. 35	—	—	14–17	7½	41½	"Clean" but "flat"	—
b	„	—	More full than (2*a*)	14–17	7½	42	—	—
3	3. xii. 35	42	Very full, inclined sour, typical "high acid" butter	14	7½	37½	"Tallowy" and rancid	4½
4*a*	7. i. 36	44½	"Sound" butter, a little flat	14	3½	44	—	½
b	„	44½	Rather flat(?) foreign flavour	14	3½	43	Slight "storage" flavour	1½
c	„	44½	Similar to (6*b*)	14	3½	42½	"Storage" flavour	2
5*a*	14. i. 36	44½	Clean flavour; slightly flat	14	3½	43½	"Sound" butter	1
b	„	44½	Rather more "flat" than (7*a*)	14	3½	43½	Rather more "full" than (7*a*)	1
c	„	44½	Rather more "full" than (7*a*)	14	3½	43½	Similar to (7*b*)	1
6*a*	21. i. 36	44½	"Clean" flavour; "sound" butter	14	3½	43½	Flavour too "full"	1
b	„	44½	(?) Slight foreign flavour	14	3½	42½	Flavour too "full"	2
c	„	44½	"Sour starter" flavour	14	3½	43	Strong flavour "like diacetyl"	1½
7*a*	25. ii. 36	44½	Natural flavour	14	4	42½	Slight "storage" flavour	2
b	„	44½	(?)	14	4	43	—	1½
c	„	44½	(?)	14	4	43	—	1½
8*a*	3. iii. 36	44½	Good quality	14	3½	43	—	1½
b	„	44½	Good quality	14	3½	43	—	1½
c	„	44	Fair	14	3½	43	Flavour too "full"	1
9*a*	10. iii. 36	44½	"Sound" but flavour rather "flat"	14	3½	42½	Slight "storage" flavour	1¾
b	„	44½	—	14	3½	43	"Full" flavour	1½
c	„	44	Flavour not as "bright" as (9*b*)	14	3½	42½	Flavour rather too "full"	1½
10*a*	26. iii. 36	44½	"Sound" butter fairly "full" flavour	14	3½	42½	Fairly "sound" butter, a little sour	2
b	„	44½	Rather "full" and soda flavour	14	3½	42	Fairly "sound", a little stale and "flat"	2½
c	„	45	Full "creamy" flavour	14	3½	43	More full in flavour than *a*, *b* or *d*. Sound butter	2
d	„	45	Fairly full "creamy" flavour and slight soda taste	14	3½	42½	Slightly sour	2½
11*a*	31. iii. 36	43½	Flavour rather "flat"	14	3½	42	Slightly sour	1½
b	„	44	More "full" than (11*a*)	14	3½	42½	Flavour fairly "full", a "sound" butter	1½
c	„	43¾	Acid, sharp, flavour	14	3½	41½	Too "full" in flavour	2¼
12*a*	2. iv. 36	44	Flavour rather harsh	14	3½	42	"Clean sour" flavour	2
b	„	44	"Full", but soda flavour	14	3½	42½	"Sound" butter "with a flavour"	1½
c	„	44½	"Nice", but too "full" to ensure food keeping properties	14	3½	43	More "full" than (12*d*), slightly sour	1½
d	„	44½	"Sound" starter butter	14	3½	42½	Fairly "full" flavour	1¾

the creams after neutralization to 0·12 and 0·15 per cent acidity has proved to be of excellent quality (series 2, 10 and 12).

In Victorian factories, the development of acidity in creams by starter before pasteurization has actually been employed as a method of increasing the flavour in "low acid" butters(6). While butter manufactured in this manner possessed a higher flavour than the ordinary "mild starter" butter, it was by no means of a "Danish" type, and tended to develop sour and harsh flavours during storage. Experiments made in this investigation confirm the findings of the Australian workers (10*a* and 12*a*).

Most butter-makers believe that it is undesirable to use starter with creams which have contained considerable amounts of acid (0·3 per cent and upwards) before neutralization, as the resulting butter will have inferior keeping properties. When, however, "high acid" cream (0·3 per cent acidity) with a "clean starter" flavour was neutralized and ripened overnight with starter to about 0·12 per cent acidity, the resulting butter was actually improved by this extra ripening process (series 2*b*, 10*c* and 12*c*).

(v) *The influence of diacetyl on the keeping quality of butter*

Effect on grade. The presence of diacetyl is considered by some authors to be detrimental to the keeping quality of butter(3, 5, 7, 8). In the foregoing section, it was concluded that diacetyl has no effect on the keeping quality of butter made from "mild starter" cream. The following results also indicate that diacetyl is not important in promoting the deterioration of butter:

Cream	Butter					
				Grading		
Churned	*p*H	Diacetyl p.p.m.	Stored at 14° F. for	Initial	Final	Loss
Finest:						
(i) Without starter	6·6	Nil	7½ months	43½	41	2½
(ii) Ripened to 0·30%	5·6	1·1	7½ months	42	37½	4½
(iii) Without starter	6·6	Nil	3½ months	44½	42½	2
(iv) 6 p.p.m. diacetyl added	6·6	1·1	3½ months	44	42½	1½

The marked deterioration of the high acid butter (ii) is related to the low *p*H value, not to its high diacetyl content.

Effect of diacetyl on carotene and vitamin A. It is usually considered that the first constituents of butter to disappear by oxidation are carotene and vitamin A. The presence of these substances in butterfat makes it of considerably more value as a food than most other fats, and certain writers are apprehensive with regard to the effect of diacetyl on the vitamin A content of butter.

The vitamin A and carotene contents were compared in butters (made from portions of the same batch of cream) after 22 months' storage under poor conditions at 14–17° F. Diacetyl had been added to two of the butters during the working stage, at the rate of 4 p.p.m., or more than twelve times the

concentration usually found in "mild starter" butters. The results are given in Table VII.

Table VII. *Loss of vitamin A and carotene in butters containing diacetyl*

Butter	Diacetyl p.p.m. Initial	Diacetyl p.p.m. Final	Vitamin A (B.U./g.) in non-saponifiable portion of fat Initial*	Vitamin A (B.U./g.) in non-saponifiable portion of fat Final	Carotene (Y.U./g.) in chloroform Initial*	Carotene (Y.U./g.) in chloroform Final	Grading remarks
1B. 8·2, finest cream	Trace	0·15 carbinol	30–35	16	30 (approx.)	20	90½ stale
1/1B. 8·2, finest cream	4	1·9 diacetyl, 0·4 carbinol	30–35	16	30 (approx.)	22	90 stale
2/2B. 8·2, finest cream + starter	4	2·2 diacetyl, 1·5 carbinol	30–35	15	30 (approx.)	22	89½ stale, unnatural diacetyl flavour

* Not determined, but known from results with similar butter.

The loss of vitamin A and its precursor carotene in both sweet-cream and "mild starter" butters containing unusually large proportions of diacetyl was no greater than found in the control butter churned from sweet cream.

These observations therefore support the view already expressed that "fullness of flavour", as determined by diacetyl content, is not harmful to the keeping quality of butter.

(vi) *The development of diacetyl in butter*

The proportion of diacetyl to carbinol in "mild starter" butter examined 1 day after manufacture is greater than in cream:

Average ratio $\frac{\text{diacetyl}}{\text{carbinol}}$ in 11 churnings in cream 0·07; in butter 0·11 (series 5, 6, 7*a*, 10*c*, *d*, 12*c*, *d*).

This observation might indicate a partition of the diacetyl with the fat, but the following results point to an actual development of carbinol in the butter:

Table VIII. *The development of diacetyl in starter butter after manufacture* (*at* 35–45° F.)

Churning No. ...	1*a*	1*b*	2*a*	2*b*	Average	
Age of butter 1 day	0·2	0·15	Nil	0·05	0·12	p.p.m. diacetyl
2 days	0·2	0·05	Nil	—	0·10	,,
3 ,,	0·3	0·2	0·1	0·1	0·08	,,
5 ,,	0·3	0·2	0·1	0·1	0·18	,,
10 ,,	0·2	—	0·1	0·2	0·17	,,
15 ,,	0·3	0·3	0·05	0·2	0·21	,,

(Series 1*b* is uneven, the butter being made by adding 16 per cent of starter to the cream just prior to churning.)

Various writers have expressed the opinion that diacetyl in butter is derived from carbinol by autoxidation with the oxygen of the air incorporated during the processes of working and churning.

This reaction has been verified by keeping a dilute aqueous solution of carbinol for 9 days, with frequent shaking, at approximately 70° F.:

	Initial	Final (after 9 days) mg.
Diacetyl/10 ml.	0·23	0·44
Carbinol/10 ml.	5·85	5·65
Ratio $\frac{\text{diacetyl}}{\text{carbinol}}$	0·04	0·08

Even under these favourable conditions, however, only one-twenty-fifth of the carbinol has been oxidized to diacetyl.

Results obtained in practice indicate that the air is not responsible for the development of diacetyl in butter.

(*a*) *The effect of reworking butter.* Butter which develops off-flavours during cold storage is usually improved considerably by reworking. This improvement, it has been suggested, is due to the formation of diacetyl, but the following results are not in agreement with this theory:

	Diacetyl p.p.m.	
Butters (10*c*, *d* and 12*c*, *d*)	Before reworking	After reworking
Reworked together after 3½ months' cold storage at 14° F. followed by a fortnight at 45° F.	0·25–0·45 (average 0·3)	0·3, 0·2 (average 0·25)

The improvement in flavour is due to other causes, the oxidation of the off-flavouring substances by the fresh air incorporated with the butter during reworking possibly being of importance.

(*b*) *The development of diacetyl in butter worked under reduced air pressure.* When starter butter is manufactured under reduced air pressure the development of diacetyl is not retarded.

			Results expressed in p.p.m. Butters kept at 40° F. for			
			7 days		25 days	
Butter	Method of manufacture	Air content % by volume	Diacetyl	Carbinol	Diacetyl	Carbinol
¼% starter added to cream before churning	Normal make	5·0	Nil	0·95	0·15	0·9
	Worked under 25 in. vacuum (by gauge)	0·1	Nil	0·95	0·15	0·8

It was therefore concluded that the changes observed in the diacetyl content of butter on keeping are unlikely to be caused by atmospheric oxidation.

Results summarized in Table V show no marked change in the diacetyl content of butter during frozen storage for 3–4 months. When the butters were held at 40° F. for 7–10 days after manufacture, the diacetyl content actually decreased in some cases.

A loss of diacetyl is not surprising, as Hammer and his collaborators (4) have shown that the lactic streptococci and numerous other types of organisms reduce diacetyl and carbinol to 2, 3-butylene glycol (a flavourless substance), particularly at *p*H values comparable with those of "mild starter" butters:

$$\underset{\text{Diacetyl}}{CH_3.CO.CO.CH_3} \xrightarrow{+2H} \underset{\text{Acetylmethylcarbinol}}{CH_3.CH_2OH.CO.CH_3} \xrightarrow{+2H} \underset{\text{2, 3-Butylene glycol}}{CH_3.CH_2OH.CH_2OH.CH_3}$$

The butters in which the most noteworthy increase in diacetyl was observed were those made from cream to which carbinol had been added, and in which the starter was growing fairly vigorously at churning (series 6).

It would appear therefore from the results described in this section that the development of the diacetyl in freshly made starter butter is merely due to the continued activity of the bacteria or their enzymes.

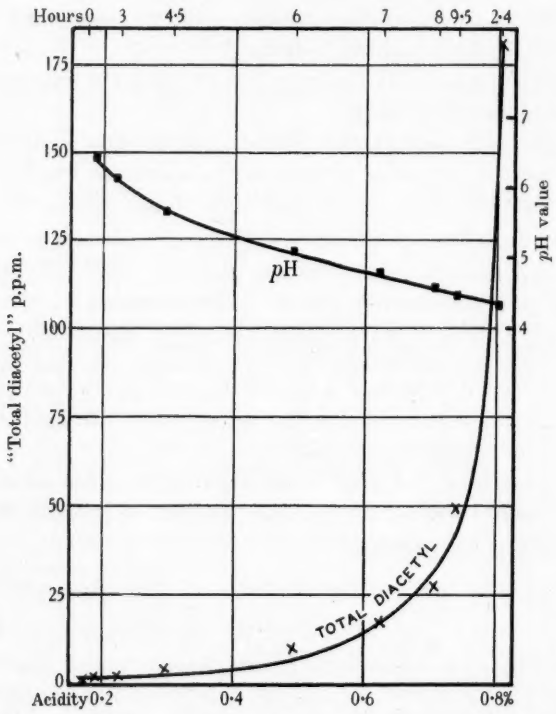

Fig. 1. The development of "total diacetyl" in a butter starter (at 30° C.).

(vii) *Experiments with starter cultures*

It is apparent that the diacetyl content of a "mild starter" butter even after a period of cold storage is, on the whole, dependent on the concentration originally present in the cream.

Hammer and his collaborators(4) have described methods for obtaining maximum flavour development in starters used for butter-making. They have also shown that the secondary (flavour) products do not appear to any extent in starter cultures until a high acidity has been attained.

The development of "total diacetyl" in a starter, which had been in satisfactory use in New Zealand butter factories for more than a year, confirms these authors' findings.

The fact that the flavour of starter butters is not necessarily proportional to the rise in acidity of the cream during ripening—in most cases the rise is due to acid added, rather than developed by the growth of the starter—is still not thoroughly appreciated by many butter-makers. For example, in a commercial butter starter (Fig. I), the amount of aroma substances (as measured by "total diacetyl" content) was, at 0·75 acidity, only about one-third of that present at 0·81 per cent acidity, after 24 hours' growth.

Starters in use at commercial factories show a considerable variation in their capacity for producing diacetyl (Table IX):

Table IX. *Development of diacetyl in commercial starters*

Starter No.	Time and temperature of growth	Final acidity when analysed	Aroma substances p.p.m.
	Cheese starters. Cultures of single streptococci		
1	20 hours at 18° C.	—	3·7 "total diacetyl"
2	20 hours at 18° C.	—	4·8 "total diacetyl"
3*a*	25 hours at 20° C.	0·83	0·2 diacetyl, 8·9 carbinol
b	25 hours at 17° C.	0·78	0·1 diacetyl, 3·2 carbinol
	Butter starters. Mixed cultures of lactic streptococci		
4*a*	24 hours at 30° C.	0·80	170 "total diacetyl"
b	Commercial factory procedure,	0·97	61 "total diacetyl"
c	i.e. 20–24 hours at approxi-	0·94	69 "total diacetyl"
d	mately 20° C.	—	82 "total diacetyl"
	Butter starters		
5*a*	Commercial factory procedure	0·95	2·7 diacetyl, 135 carbinol
b		—	2·3 diacetyl
c		0·90	2·1 diacetyl, 80 carbinol
d		1·02	3·0 diacetyl
6*a*	Commercial factory procedure	0·86	5·0 diacetyl, 104 carbinol
b		0·76	5·5 diacetyl, 95 carbinol
c		0·92	3·9 diacetyl, 126 carbinol
d		0·86	4·1 diacetyl, 104 carbinol

With butter starters, there is no proportionality between the final acidity and the content of diacetyl and carbinol. The higher amounts of diacetyl are, on the whole, found in starters which have ceased growing at relatively low acidities (see also Table X).

An investigation was also made on the effects of (1) the period of ripening, and (2) the medium of growth, on the development of diacetyl and carbinol. The results, shown in Table X, were obtained with milk or cream, steamed for 30–40 min., allowed to cool and inoculated with 0·5 per cent starter.

The results indicate that there is no essential difference, when due allowance is made for the fat content of the cream, in the action of the starter, whether grown in skim milk, whole milk, or cream.

The unusually small production of "total diacetyl" in a starter grown at a rather lower temperature than usual is shown by the series (6 *k–m*, Table X). Conversely, increased amounts of "total diacetyl" are obtained at higher temperatures (4*a*, Table IX).

It will be seen that starters prepared in butter factories are not always at their stage of maximum flavour development when used. The practice of keeping ripened starters for a day at chilled temperatures, as followed in

Table X. *Development of diacetyl by starters under various conditions of growth*

Starter No.	Medium	Temperature ° C.	Period of ripening hours	Final acidity %	p.p.m. Diacetyl	p.p.m. Carbinol
6*e*	Skim milk	20	20	0·85	4·3	—
f	Whole milk	20	20	0·82	5·2	—
g	Cream	20	20	0·46	1·8	—
h	Whole milk	20	21½	0·82	7·1	77
i	Whole milk 5% starter	20	21	0·89	5·5	100
j	Whole milk	20	22½	0·90	5·3	87
k	Skim milk	18	20	0·85	0·8	22
			44	0·96	1·9	187
l	Whole milk	18	20	0·81	1·3	19
			44	0·95	2·4	172
m	Cream	18	20	0·39	0·6	14
			44	0·55	4·9	59

certain American factories (4), would appear to be a sound modification of the present procedure.

Attempts to convert carbinol to diacetyl by means of lactic streptococci. The close chemical relationship between carbinol and diacetyl and the fact that, in milk products, the carbinol is present in considerably greater amounts than the diacetyl have led to the assumption that the carbinol is the precursor of diacetyl.

Diacetyl develops in starter butters when held at 40° F., but experiments detailed elsewhere indicate that the change is not due, as has been suggested, to oxidation by air.

Virtanen & Tarnanen (12) consider, however, that oxygen is necessary for the formation of diacetyl in the growing starter, but results obtained by the author are not in agreement with their theory: (i) Ripe butter starter (skim milk) was shaken with air in glass vessels (*a*) alone, (*b*) with 10 per cent sodium chloride (simulating butter serum), and (*c*) with 10 per cent sodium chloride and 15 p.p.m. Fe″ (sulphate) added as an oxidation catalyst. Control samples were prepared and left unshaken near the others. No consistent increase in the diacetyl content was noted on the four occasions the experiment was made, in fact the air tended to destroy the diacetyl. (ii) A starter was grown in whole milk at 21° C. for 70 hours, one portion being shaken with air, the other being undisturbed. The aeration repressed the production of diacetyl to a very marked extent:

Starter	Acidity %	p.p.m. Diacetyl	p.p.m. Carbinol
Aerated by shaking	0·82	0·3	1·9
Unshaken control	0·91	1·7	72

The production of diacetyl by bacterial dehydrogenases. When the diacetyl content of butter increases, there is not a corresponding increase in the

carbinol content (see in particular, Churning series 6, Tables I and II). The diacetyl is therefore possibly derived from the carbinol and the change may be due to bacterial dehydrogenation. A striking parallelism exists between a suggested mechanism for the conversion of carbinol to diacetyl, and the well-established dehydrogenation by bacteria of lactic to pyruvic acid:

$$\underset{\text{Acetylmethylcarbinol}}{H_3C.CHOH.CO.CH_3} \xrightarrow{-2H} \underset{\text{Diacetyl}}{H_3C.CO.CO.OH}$$

$$\underset{\text{Lactic acid}}{H_3C.CHOH.CO.OH} \xrightarrow{-2H} \underset{\text{Pyruvic acid}}{H_3C.CO.CO.OH}$$

Hammer and his collaborators(4) cite various references in the literature and some experiments of their own to show that the precursor substance 2, 3-butylene glycol is dehydrogenated to carbinol when biologically activated by resting bacteria.

It was not possible for the author to apply the Thunberg technique, and experiments made with starters under practical conditions have failed to yield any proof of the reaction suggested. A brief summary only of the results will be given:

(1) Butter starter was grown in milk containing as precursor substances (*a*) 10 per cent neutralized, pasteurized starter; (*b*) 20 p.p.m. carbinol; (*c*) 100 p.p.m. 2, 3-butylene glycol. There was no evidence of increased diacetyl production due to the presence of carbinol or glycol.

(2) Milk was inoculated with butter starter, and when the acidity had attained 0·25 per cent (equivalent to a rise of 0·05 per cent acidity in cream) it was divided among three vessels, 10 per cent of salt being added to each, to simulate butter serum. One of the milks was used as a control, to another was added 20 p.p.m. carbinol, and to the third 100 p.p.m. of dibutylene glycol. The samples were left for 21 hours at 20° C., the acidities rising to 0·27 per cent. No evidence of diacetyl production from the precursors was found, and the carbinol content of the milk to which this substance was added had actually fallen to about one-half of the initial value.

A similar result was obtained in a series of tests with a culture of a single strain of a lactic streptococcus, in commercial use as a cheese starter: carbinol (20 p.p.m.), 2, 3-butylene glycol (100 p.p.m.) and diacetyl (5 p.p.m.) were added to milks with the starter. After 24 hours' growth at 20° C. the diacetyl in (*c*) had decreased to 4·5 p.p.m., and the carbinol content in (*a*) had fallen to one-half the original value. There was no evidence of diacetyl production in any of the samples.

While it has not been possible to prove by the simple tests described that the carbinol is converted to diacetyl by the dehydrogenase activity of lactic streptococci, the bulk of the evidence in this paper—particularly in section VI—is consistent with this theory. The alternative theory—that the diacetyl is derived from the carbinol by oxidation with oxygen of the air—is not in accord with the observations.

SUMMARY

1. Acetylmethylcarbinol in cream is partly destroyed by neutralization and pasteurization, but the diacetyl content is practically unchanged.

2. Diacetyl and carbinol are retained in butter with the butter-milk fraction.

3. Diacetyl is present in butter: (*a*) sweet-cream 0·05 p.p.m., (*b*) "mild starter" 0·3–0·4 p.p.m., and (*c*) "high acid" 1·5 p.p.m. The diacetyl content remains fairly constant during 3½ months' storage at 14° F.

4. Diacetyl in butter does not affect the keeping quality or vitamin A content.

5. The diacetyl content tends to increase when butter is held at 40° F. for several days after manufacture. The change is not caused by oxidation with air, but is probably due to dehydrogenase activity of the "starter" organisms or their enzymes.

6. Experiments with butter "starters" are described.

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149. THE COAGULATION OF MILK WITH RENNET. SOME EXPERIMENTS WITH SLOW-RENNETING AND SOFT-CURD MILKS

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(With 7 Figures)

Reference has frequently been made in the literature to difficulties experienced by cheese-makers in obtaining a satisfactory rennet coagulation with some milks. It is well known that temperature of coagulation and previous heat treatment of the milk have very important effects on the rate of coagulation and type of coagulum obtained. In the manufacture of Cheddar cheese, however, the permissible range of temperature of setting with rennet is very limited (86–90° F.); and in this country the heat treatment given to the milk, under present conditions of flash pasteurization at temperatures not over 160° F., is not sufficient to affect coagulation with rennet to any appreciable extent. The work herein described, therefore, is concerned for the most part with the relationship of individuality of milk to the renneting time and the type of coagulation.

In 1908 Van Dam (1) quoted an instance of a cow giving milk which would not curdle with rennet, and claimed that the defect had been overcome by the daily addition of 50 g. of calcium diacid phosphate to the ration. Koestler (2) summarized the results of a detailed examination of a series of milks which would not give a satisfactory coagulation with rennet and divided these "slow renneting" into three types:

Milk type A. Salty milk, which was characterized by high chlorine content, low lactose content, low acidity, and high albumen content, and was attributed to the presence of inflammation of the udder—in other words, what is now termed "mastitis" milk. The curdling time was shortened but the coagulation obtained was soft and unsatisfactory. Increase of acidity, or addition of calcium chloride, or an increase in the quantity of rennet proved satisfactory correctives. (Milk from many cows at the end of the lactation period has somewhat similar properties to the "mastitis" milk.)

Milk type B. This type was considered to be of very rare occurrence. Rennet under the usual conditions gave no apparent reaction, i.e. no coagulation, but coagulation took place in the milk to which rennet had been added, immediately on the addition of some calcium chloride. The chemical composition of the milk was normal.

Milk type C. The milk coagulated with rennet in normal time, but the coagulum did not gel properly. The gel remained for a relatively long time in a soft semi-liquid state. This milk also was normal in composition.

Koestler considered that milk of type C was the most dangerous, as it could "infect" normal milk with its non-curdling properties. In a later publication (3), however, he grouped milks of types B and C into one class, since he was not able to establish a sharp line of demarcation between them. In his paper a large number of instances are given where trouble in the coagulation of cheese milk and in the proper drying out of the curd was experienced, because of the inclusion in the general supply of slow renneting milks from a few cows in the herds of one or more of the suppliers. Removal of these milks from the bulk supply eliminated the trouble.

In New Zealand difficulties with the coagulation of vat milk in cheese factories have occurred spasmodically during the past few years. Chemical analysis of the milk has failed to reveal any abnormality, and the acidity and casein content have been normal. The milks encountered have belonged more to Koestler's type C than to type B, i.e. a flocculent coagulation could be obtained in the normal time but the curd would not "firm-up" properly. Even the adoption of a longer ripening period, in order to increase the acidity at renneting, did not "firm-up" the curd, although it decreased the time of formation of the flocculent coagulum, in agreement with Koestler's findings with this type of milk. The addition of calcium chloride caused a reduction in renneting time and a "firming" of the curd, but the proportion of calcium salt required was quite considerable. For 50 ml. milk at least 0·2 ml. $M/2$ calcium chloride solution was necessary, but for a really satisfactory curdling of the milk 0·4 ml. was required. This was equivalent to an increase of 0·011–0·022 in the percentage of CaO in the milk, i.e. a 6–12 per cent increase. As already pointed out by Koestler, such additions to milk for cheese-making bring further problems to the cheese-maker. Koestler recommends the elimination of the abnormal milk from the cheese vat.

In the investigations carried out in New Zealand it has not been possible to follow the system of Koestler in examining the individual supplies of milk to the factory and then to follow up the matter by examination of the milks of the separate cows in the offending herds. There appear to be some differences, however, in the experiences of the trouble in Switzerland and in New Zealand. It is usually found in the latter country that the trouble, when it appears, affects all the vats simultaneously. In the one factory where the individual herd supplies were tested, it was found that ten out of the fifteen supplies were of the soft curd type. The cause of the trouble has not been defined, but the occurrence is frequently simultaneous with the occurrence of drought. In view of the localization of the trouble to small areas, and the generally more widespread occurrences of drought, it is apparent that there is some further additional contributing cause.

From another point of view, viz. its importance to the use of cow's milk

in infant nutrition, the subject of coagulation of milk has recently received a great deal of attention in the United States of America. Following on the work of Buckley(4) and of Alleman & Schmid(5) in developing a method of measuring the strength of milk curd, Hill(6) has patented an instrument called the Curd-o-meter, which gives a measure, in grams, of the resistance of the curd to fission by a series of radiating blades, and which has been used widely as a basis for the choice of milks for the feeding of infants. Modifications of the apparatus and of its method of application have been made by Sommer & Matsen(7) and by Cole(8), but the principle used is unaltered. Hill used as a coagulant a solution of pepsin containing calcium chloride, on the basis that pepsin is the chief coagulant in the infant stomach; and because the preparation of a standard solution of pepsin was more easily realizable than the preparation and preservation at constant strength of a standard rennet. The addition of calcium chloride was claimed to give a sharper differentiation between milks of the "hard" curd and "soft" curd types. The workers in the field have shown that there are wide variations in the curd tension of the milks from individual cows. Milks with curd tensions of less than 30 g. have been classed as "soft-curd" milks. Endeavours to relate the softness of curd to some causal factor in the milk have not been very successful. Weissberg *et al.*(9), for example, found no relation between curd tension and whey protein concentration, lactose, citric acid or ash content of the milk, or any of the whey constituents; or viscosity; or the *p*H of the individual milks (although differences were, of course, observed for any one milk at different *p*H's). The soft-curd milks are more clearly correlated with casein content than with anything else, although here also the correlation is not regular. There are many cases of milks of low casein content and high curd tension and of milks with high casein content and low curd tension. The generalization holds, however, in the main, that the soft-curd milks are produced by cows of the low testing breeds, viz. Friesian and Ayrshire (Hill(6) and also a number of other workers). Whether the low curd tension is due actually to the low casein content is still not proved. The fact that milks of the same casein content frequently give widely different types of curds shows conclusively that the "soft curd" property of a milk is due in part at any rate to some peculiarity in the constitution or physical state of the milk apart from any peculiarity of composition. The finding of Koestler (3), that the ultra-microscopic picture of slow-renneting milks differed from that of normal milk in that more of the larger protein particles were present, has not been related to Hill's classification of milks.

It has been shown by many workers that the incidence of mastitis causes a reduction in the curd tension of milk (Koestler's "rasssalzige" milk). Sommer & Matsen(7) have reviewed the evidence on that question, and have demonstrated the effect of mastitis in individual infected quarters in lowering the curd tension for that quarter. The average curd tension of the milk from a herd of thirty healthy cows was 46·8 g., renneting time 7·6 min., compared with 37·9 g. and 10·6 min. for a herd of fifteen infected cows. (Their comparison

is vitiated to some extent by the fact that the proportion of Friesian and Ayrshire cows was only 39 per cent in the non-infected herd compared with 53 per cent for the infected herd.) The lowering of curd tension in milk from infected quarters was confirmed by Welch & Doan(10) (see also Sanders *et al.*(11)). Hansen *et al.*(12) found that there was a lowering of curd tension with streptococcic infection but not with a staphylococcic infection.

Milk can be reduced in curd tension by homogenization (Berry(13), Theophilus *et al.*(14) and Courtney(15)); by passage over an appropriate zeolite, which effects a 20 per cent lowering of the calcium content of the milk (Lyman *et al.*(16)); by the application of intense sonic vibration (Chambers(17)), or by churning at a low temperature (Lundstedt(18)).

The information available on the relationship of Hill's soft-curd milk to cheese-making is very meagre. Hill & Merrill(19), on the basis of some experimental comparisons, claim to have shown that hard-curd milk is more suitable for cheese-making than soft-curd milk, both on account of the quality of the cheese, and because the losses of fat and protein in the whey were lower. The general inferior quality of their experimental cheese, and the very high fat and protein losses recorded for both types of milk indicate that the cheese-making technique was greatly at fault, and the results cannot be accepted as significant.

From the point of view of the examination of the milks to be used for cheese-making, Hill's system of producing the coagulation of milk with pepsin and calcium chloride must be regarded as artificial and unsuitable. As has already been stated, Koestler(2) has shown that some milks respond much more readily to the addition of calcium chloride than others. The use by Hill of such large amounts of calcium chloride in the pepsin coagulant therefore could lead to erroneous results. Caulfield & Riddell(20) found the test unsuitable for examination of milks for the manufacture of Swiss cheese, and Sanders *et al.*(11) formed the same conclusion for Cheddar cheese.

Experimental

The work on the renneting of milk was begun in 1930 following the first case of coagulation trouble in a cheese factory to be reported to this Institute. In agreement with the conclusions of Koestler(2) for milks of his types B and C, it has not been possible to find any relationship between the composition of milk, more particularly the calcium and phosphorus content, or the Ca/P ratio, and the type of curd obtained; nor has any causal factor for the sudden appearance of the trouble in all vats in a factory been discovered. Hence the work herein detailed is for the most part descriptive.

Methods

(1) *Measurement of coagulation or renneting time*

Morning's milk was used. The sample (50 ml.) was measured with a quick delivery pipette into a 100 ml. beaker and was warmed to 30° C. with careful stirring. The beaker was then immersed to the level of the milk in a water bath at 30° C., 1 ml. of freshly diluted rennet (1 part commercial liquid rennet to 2 parts water) added, and the beaker gently rocked until a flocculent coagulum appeared, the time from addition of rennet being taken with a stopwatch.

The method differs from that of Koestler in two particulars:

(*a*) *The temperature of renneting.* Koestler was examining the milks from the point of view of the manufacture of Swiss cheese, and chose the temperature 35° C. for his test, whereas for Cheddar cheese 86–90° F. (30–32° C.) is the normal setting temperature.

(*b*) *The quantity of rennet added.* Koestler added sufficient rennet to cause flocculation in normal milk in about 10 min., the time usually taken in the Swiss cheese vat. The present method involves the addition of much greater quantities of rennet than are used in the cheese vat, viz. 1 part of liquid rennet per 300 parts of milk instead of the normal 3 oz. per 100 gallons or 1 part in 5000, the object being to speed up the experimental work. For comparative purposes the method gives satisfactory results. The effect of the increased quantity of rennet is to harden the curd, and cause coagulation which under exact cheese-making conditions might not occur.

(2) *Classification of types of curd*

Immediately after commencement of flocculation, in the estimation of renneting time, the rocking of the beaker was discontinued and the curd allowed to stand at room temperature for 15–30 min. The curds were then examined independently by two observers. For the examination the curds were shaken in the beaker, and the manner in which the coagulum separated from the walls of the beaker, the manner in which it held together, and the type of vibration in the gel when shaken were noted. On the basis of these observations the curds were classified into the following types, which were later given the arbitrary numerical values shown:

(1) Very firm (V.F.)	points allotted	10
(2) Firm (F.)	„	8
(3) Moderately firm (M.F.) ...	„	6
(4) Moderately soft (M.S.) ...	„	4
(5) Soft (S.)	„	2
(6) Very soft (V.S.)	„	0

It was found that two workers after a little practice could make the classification with a surprising degree of concordance. It frequently happened

that, out of 100 independent examinations by two observers, the classification of the curds differed by as much as two stages in the above scale in only two or three cases. These cases were always re-examined until an agreement was reached with regard to the classification.[1]

Results

A monthly examination of milks from the Massey Agricultural College herd of 100–120 cows over the years 1931–2, 1932–3 and 1933–4 was made at the same time as the monthly herd-test examination for fat production. The cows were grazing throughout the year, but received supplementary feeding of ensilage, hay or turnips during the autumn and winter. The herd was made up, for the most part, of spring and autumn calvers. All milks examined were free from mastitis but a few of the cows in the herd had previous histories of mastitis. The results for the three breeds, viz. Friesian, Ayrshire and Jersey, are given separately.

(1) *Relation between period of lactation and renneting time*

The results for each month of lactation, averaged for cows of each of the three breeds, and the corresponding number of results are shown in Table I. The renneting time is plotted against month of lactation in Fig. 1. It will be seen that with all breeds the renneting time tended to rise with progress of lactation, but this was particularly marked with the Ayrshire milk which at the end of the lactation, on the average, took twice as long to coagulate as at

Table I. *Number of results and average renneting times of Friesian, Ayrshire and Jersey milks, monthly throughout the lactation period*

	Friesian		Ayrshire		Jersey	
Month of lactation	No. of results	Average renneting time (sec.)	No. of results	Average renneting time (sec.)	No. of results	Average renneting time (sec.)
1	93	49·6	55	56·8	144	45·1
2	96	54·9	57	66·4	117	46·7
3	98	57·4	56	82·8	115	47·5
4	98	61·3	56	80·3	118	46·4
5	99	61·7	57	85·6	118	46·5
6	101	61·4	56	90·3	116	47·2
7	99	63·5	54	107·3	115	50·0
8	98	62·6	41	101·2	110	64·3
9	88	82·6	31	109·4	91	64·3
10	45	59·9	17	110·1	51	61·9

[1] The above system of classification was developed independently of the system of Koestler (18), which did not come to the authors' notice until this paper was in preparation. Koestler used a somewhat similar method of examination, but he kept the coagulated samples at the temperature of coagulation, he specified more accurately the true interval, viz. 10 min., between observation of flocculation and examination for curd type, and he described only five types of coagulation, viz. very good, good, somewhat unsatisfactory, unsatisfactory and very unsatisfactory. In addition to the criteria used by the present authors, he made an observation of the milkiness of the separating whey. His method was therefore more precise than that used in the present investigation.

the beginning. The average renneting time of the Ayrshire milk was consistently much higher than that of Friesian or Jersey milk, and the average renneting time of Friesian milk was higher than that of Jersey milk.

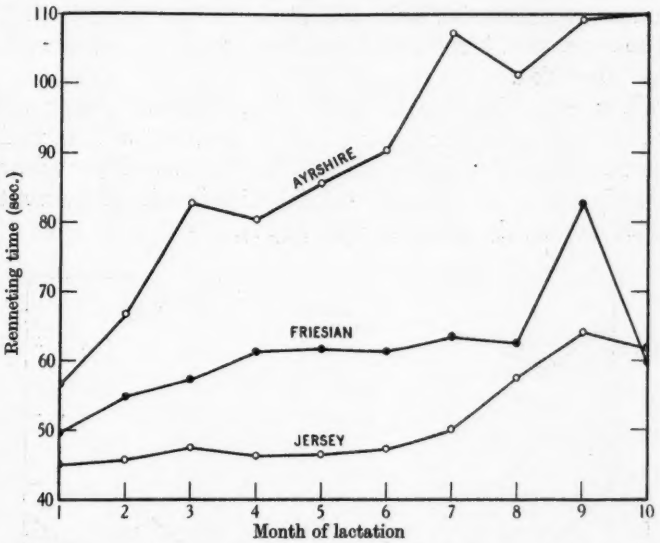

Fig. 1. Average values of renneting times for milks of Friesian, Ayrshire and Jersey cows at different stages of lactation.

(2) *Relation between type of curd and period of lactation*

The curd classification results converted to the numerical form for the milks of cows of the three breeds were averaged for each breed. Where two observers differed in their classification by one stage, e.g. M.F. (6 points) and F. (8 points), the points for these two classes were averaged. The results are given in Table II and are plotted in Fig. 2.

Table II. *Number of results and average "firmness" of curd for Friesian, Ayrshire and Jersey milks, monthly throughout the period*

	Friesian		Ayrshire		Jersey	
Month of lactation	No. of results	Average curd firmness	No. of results	Average curd firmness	No. of results	Average curd firmness
1	92	5·1	55	6·0	114	8·4
2	95	3·9	57	5·1	117	7·7
3	98	4·2	56	4·4	115	8·1
4	98	4·5	56	5·2	118	8·4
5	99	4·5	57	4·7	118	8·5
6	101	4·8	56	5·1	116	8·6
7	99	4·9	54	4·7	115	8·9
8	98	5·1	41	5·5	110	8·6
9	88	4·9	31	5·1	91	8·0
10	45	5·6	17	5·3	51	8·4

With milk of all three breeds there was in every case a decrease in firmness of curd at the beginning of lactation. This is in agreement with the findings of other workers for the Hill curd test (compare Berry (13), Welch & Doan (10)). The indication that the fall in curd strength with Ayrshire milk continues into the third month of lactation is also in agreement with other findings. On the whole, however, there is not a very significant change in curd strength with progress of lactation.

It will be noted also that the Jersey milk gave much stronger curds, on the average, than Friesian or Ayrshire milk, in agreement with the findings of other workers for the Hill curd tension test. The Ayrshire milk, on the average, gave slightly firmer curds than the Friesian milk, in spite of the fact that it coagulated more slowly with rennet (see Fig. 1).

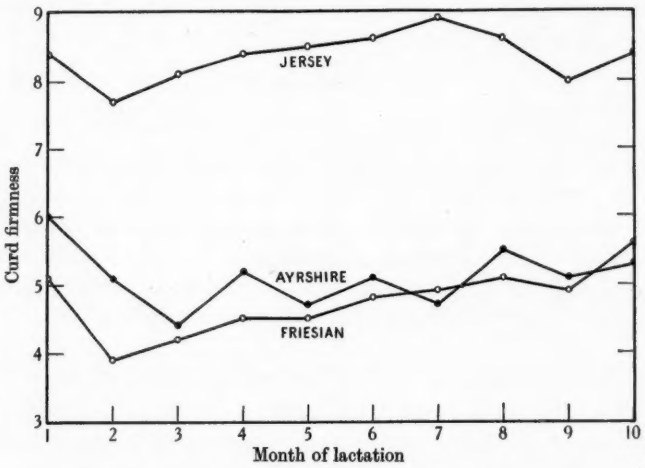

Fig. 2. Average curd firmness of milks of Friesian, Ayrshire and Jersey cows at different stages of lactation.

(3) *Relation of composition of milk to curd type*

This subject is of interest in view of the conclusions of other authors that the curd strength as determined by the Hill test is related to the casein content of the milk, and of a similar conclusion reached by Sanders *et al.* (11) for the rennet curd. Unfortunately values for the casein contents of the milks are not available, but in view of the average relationship between fat and casein in milk it may be assumed that a rise in fat content usually signifies a rise in casein content. The fat contents of the milks yielding curds of different types have been classified according to the curd type on the scale 1–10. The number of results and the average fat contents taken out for each type for the milks of the three breeds of cows are given in Table III, and the results are plotted in Fig. 3.

Table III. *Number of results and average fat content of milks giving curds of various degrees of firmness for the Friesian, Ayrshire and Jersey breeds*

	Friesian		Ayrshire		Jersey	
Curd firmness	No. of results	Average fat content	No. of results	Average fat content	No. of results	Average fat content
0	65	3·50	36	4·89	3	5·40
1	45	3·34	17	4·56	2	4·95
2	195	3·59	64	4·38	10	5·86
3	91	3·36	34	4·33	7	4·64
4	186	3·69	83	4·50	43	5·31
5	83	3·63	42	4·39	28	5·54
6	148	3·75	91	4·55	130	5·45
7	60	3·62	42	4·76	89	5·07
8	95	4·05	58	5·05	337	5·49
9	22	4·00	15	4·85	135	5·25
10	32	4·34	25	5·42	355	5·76

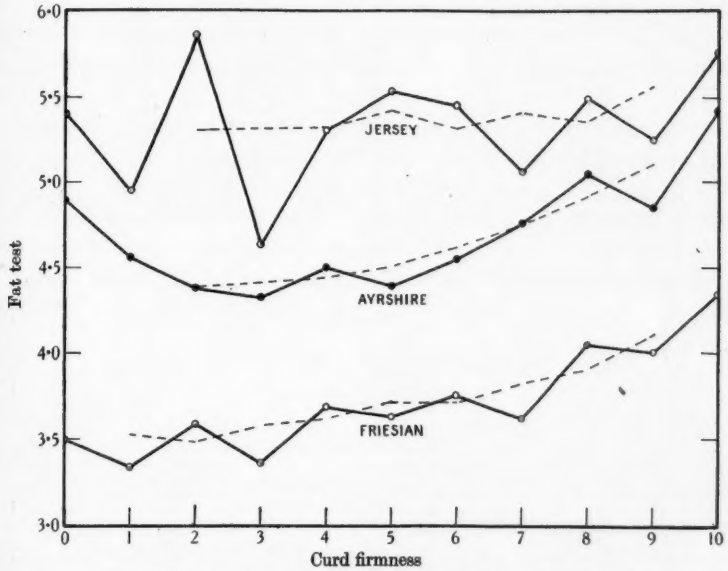

Fig. 3. Relationship between curd firmness and fat test of milk of Friesian, Ayrshire and Jersey cows.

Account has not been taken of period of lactation, but the results given above in Table II and Fig. 2 show that this would not introduce any considerable error. It is evident from Fig. 3 that the relationship of fat content of milk to curd type is dependent on the breed of cow. (The dotted lines in the figure representing the moving averages taken out over three sets of results show the general tendency more clearly.) With Jersey cows the curd firmness is independent of the fat content of the milk. With Friesian and Ayrshire milk there is apparent a definite increase in curd firmness with increasing fat content of the milk, amounting on the average to an increase of 0·6 per cent fat in the milk over the range from "very soft" to "very firm" curds.

(4) *Relation between renneting time and curd type*

Renneting times were averaged for milks of each curd type on the scale 1–10. The number of results and the averages are given in Table IV and Fig. 4.

Table IV. *Number of results and average renneting time of milks giving curds of various degrees of firmness for the Friesian, Ayrshire and Jersey breeds**

	Friesian		Ayrshire		Jersey	
Curd type	No. of results	Average renneting time (sec.)	No. of results	Average renneting time (sec.)	No. of results	Average renneting time (sec.)
0	83	99·1	36	184·1	8	192·0
1	56	89·3	19	134·7	4	111·7
2	216	59·5	66	109·8	11	107·3
3	104	60·3	34	83·3	12	83·8
4	214	61·8	89	80·6	50	74·8
5	108	55·3	46	73·5	31	68·0
6	164	56·3	94	69·5	142	61·6
7	65	43·4	50	58·3	113	48·5
8	127	55·6	65	63·6	387	52·3
9	24	42·8	16	48·4	164	43·4
10	34	54·4	27	55·7	364	46·4

* In all cases where the renneting time of a sample was longer than 240 sec. it has been recorded as 240 sec. for calculation of the averages.

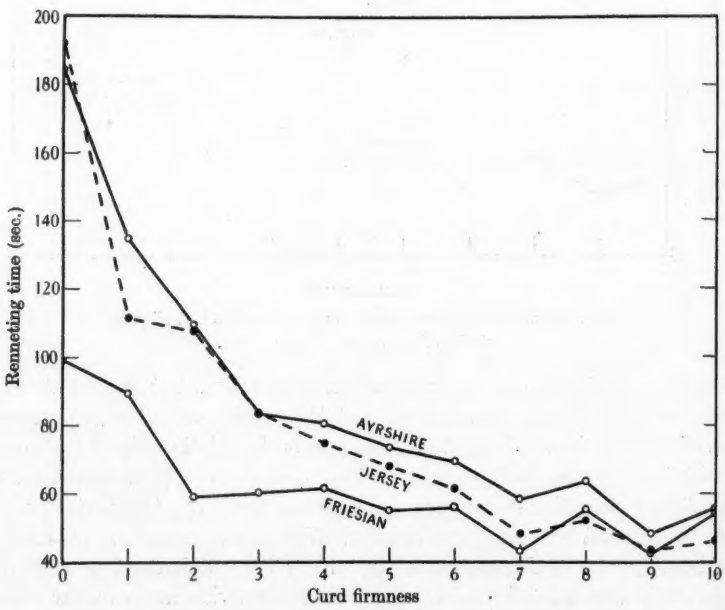

Fig. 4. Relationship between curd firmness and renneting time of milk of Friesian, Ayrshire and Jersey cows.

It is evident:

(*a*) that soft-curd milks give longer renneting times than hard-curd milks, for all three breeds of cows;

(*b*) that the effect was shown more markedly by Ayrshire and Jersey milks than by Friesian milks.

Effect of dilution and concentration of milk on renneting time and curd type.

In view of the conclusions of other workers that the firmness of curd is related to the casein content of the milk, it is of interest to study the effect of dilution of milk with water, and of concentration of milk, on this property and on the renneting time.

(1) *Dilution.* Van Slyke & Price (21) state that at least 10 per cent of water may be added to milk without affecting its renneting properties, but give no references to the source of this information. Lind (22) found a progressive increase in renneting time with increase in dilution with water, but this effect was not very marked up to the addition of 10 per cent of water. His results are cited in Table V.

Welch & Doan (10) concluded that dilution of milk with water up to 30–40 per cent reduced curd tension in proportion to the reduction in casein content, but beyond this point there was little change.

The following results show that with some milks the dilution may be much greater than 10 per cent without seriously affecting the curd. Hence the effect of added water on the fat loss in cheese whey will depend on the type of milk being used (compare McDowall (23), pt. 2).

Table V. *Effect of addition of water on renneting time and curd type of milk*

Water added %	Milk A		Milk B		Results of Lind (22).
	Renneting time (sec.)	Curd firmness	Renneting time (sec.)	Curd firmness	Coagulation time (min.)
0	59	10	42	8	7·3
5	59	8	40	8	7·5
10	63	6	42	8	7·9
15	63	6	44	8	—
20	64	6	45	6	8·7
30	66	4	45	4	9·8

(2) *Concentration.* Ordinary evaporated milk usually has a soft curd when reconstituted, because of the effect of heat on the curd coagulation (Hill (6)).

The authors' experimental work was carried out in two ways:

(*a*) A volume of milk was evaporated under reduced pressure at 20–30° C. At intervals the volume remaining was measured, and a sample was withdrawn for test, the evaporation being continued with the remainder. The results, grouped together in sequence of concentration in Table VI and curve 1 of Fig. 5, were obtained from evaporation of two separate portions of a sample of milk. It will be seen from the smoothness of the curve that the effect was consistently shown in the duplicate experiments.

Table VI. *Effect of progressive concentration of milk on renneting time, and on curd type.* (*Original milk of a soft-curd type*)

Volume of milk and percentage of original volume	Renneting time (sec.)	Type of coagulum	Curd firmness
100	93	Indefinite	2
99	103	,,	2
92	107	,,	2
82·6	95	,,	4
80·9	93	,,	4
73·9	84	Fair	6
65·4	28	,,	6
63·1	78	,,	6
57·7	77	,,	6

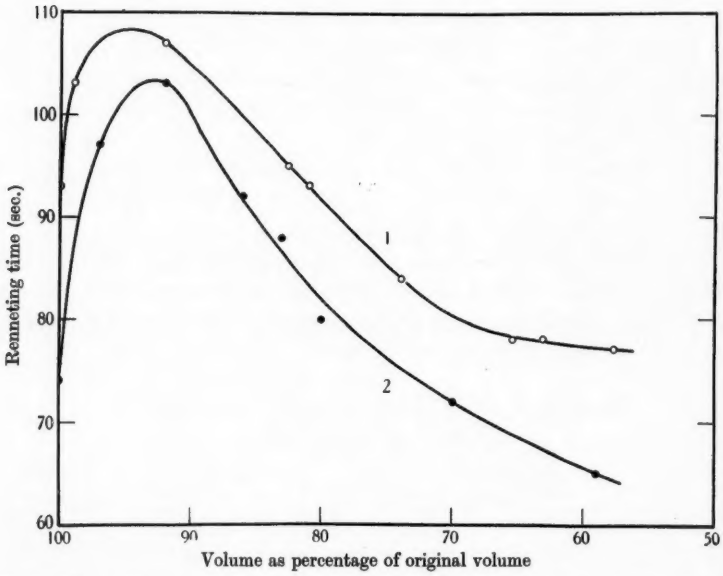

Fig. 5. Effect of concentration of milk in renneting time. Curve 1, progressive evaporation of one sample. Curve 2, evaporation of separate samples to different concentrations.

Concentration of milk in vacuum thus gave an increase in renneting time initially, probably due to loss of carbon dioxide. With increased concentration of solids there was a steady fall to a value somewhat below that of the original milk. The resultant curd became progressively firmer but at the same time lost the smooth character of the original curd. Concentration to less than 60 per cent of the original value did not increase the hardness of the curd beyond the stage of "moderately firm" (6 points).

(*b*) The method (*a*) involved heating of the later portions of the milk for a considerable time, which conceivably could affect the coagulation with rennet. In method (*b*) separate quantities of 200–260 ml. of milk were evaporated to various volumes, the volume of residual milk was measured and

a sample was submitted to the rennet test. The results are given in Table VII and in curve 2 of Fig. 5.

Table VII. *Effect of evaporation of milk on time of coagulation with rennet and on type of curd*

Volume of milk as percentage of original volume	pH of milk	Renneting time (sec.)	Type of coagulum	Curd firmness
100	6·73	74	Indefinite	2
97	6·69	97	,,	2
92	6·73	103	,,	4
86	6·73	92	Fair	4
83	6·68	88	,,	6
80	6·73	80	,,	6
70	6·69	72	,,	8
59	6·66	65	,,	10

The results show again the initial rise in renneting time with increase in concentration, and then a gradual fall, with increasing concentration, to a value below that of the original milk.

Effect of mixing "hard"- and "soft"-curd milks.

The finding of Koestler(2) that some slow-renneting milks can "infect" normal milk implies that a small amount of soft-curd, or slow-renneting, milk can influence the renneting time or curd type of normal milk to a considerably greater extent than would be expected from the proportion added. It seemed of interest therefore to study the effect of mixing the two types of milk. Results from several experiments are given in Table VIII and in Fig. 6.

Table VIII. *Renneting time and types of curd from admixtures of various proportions of slow-renneting milk with quick-renneting milk*

% slow-renneting milk	Exp. 1		Exp. 2		Exp. 3	
	Renneting time (sec.)	Curd firmness	Renneting time (sec.)	Curd firmness	Renneting time (sec.)	Curd firmness
0	42	8	41	10	61	10
10	43	6	44	10	62	10
20	48	4	46	8	67	10
30	53	4	52	8	74	8
40	54	4	54	8	91	8
50	57	4	61	8	110	6
60	63	4	66	6	133	4
70	68	4	72	6	156	4
80	78	4	83	4	165	2
90	85	4	92	4	200	2
95	94	2	100	4	>240 no coagulation	0
100	107	2	104	2	>240 no coagulation	0

It will be seen that in every case the curve is parabolic whereas if the effects of the two types of milk on each other were purely additive, the curve should be linear. Furthermore, the slope of the curve in every case is greatest when the proportion of slow-renneting milk is greatest, showing that the tendency was for the "hard-curd" quick-renneting milk to impose its properties on the

"soft-curd" slow-renneting milk, and not the reverse as found by Koestler. This was particularly noticeable in Fig. 6. In this case the soft-curd milk did not coagulate in 30 min., but with 10 per cent of quick-renneting milk a definite coagulation was obtained in 30 sec.

From this point of view it is of interest to study more closely the results of Koestler. There was in some cases a definite indication of the "infecting power" of a mixture containing 7·5 per cent of slow-renneting milk, but this is scarcely important enough to be called an "infection". The results are in

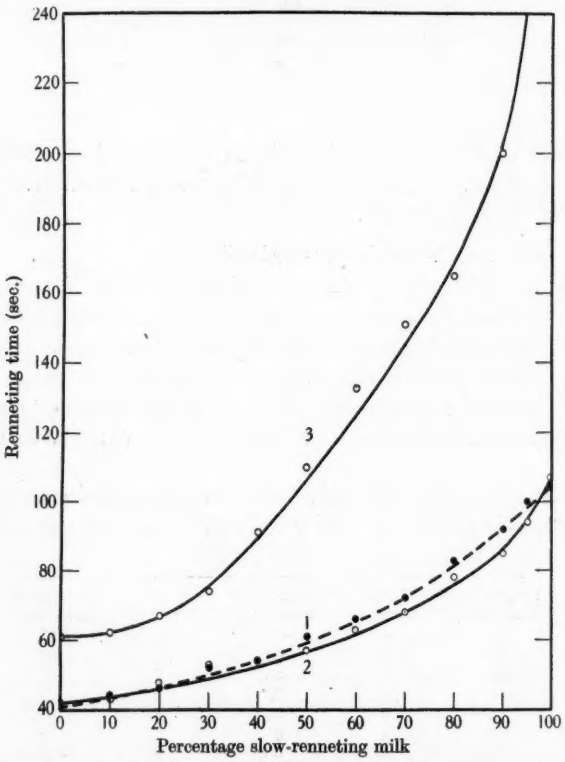

Fig. 6. Effect of mixing hard- and soft-curd milk on the renneting time.

agreement with those cited by the present authors, in that they indicate very little change in renneting time or curd type through admixtures of small quantities (e.g. 10 or 20 per cent) of slow-renneting milk with the quick-renneting types. With mixtures containing 80 per cent slow-renneting milk Koestler found on occasions an increase in the renneting time over that for the slow-renneting milk alone. It is possible, however, that Koestler's references to infection are concerned more with the effect of the milk on the gel and on the general cheese-making procedure, and in this respect his conclusions are borne out by the results from his publication quoted above.

The findings of the present authors have been confirmed in two ways:

(1) The renneting time of accurately prepared composite samples of several milks was found always to be lower than the averages of the renneting times for the individual milks, e.g. seven quick-renneting milks gave an average renneting time of 46 sec., and the composite coagulated in 42 sec. The corresponding figures for seven slow-renneting milks were 139 and 107 sec. respectively.

(2) Addition of 20 per cent of various quick-renneting milks to each of a number of slow-renneting milks from individual cows invariably reduced the renneting time of the latter below the time calculated on the basis of a simple proportional relationship.

Effect of addition of hydrochloric acid and calcium chloride on the coagulation of slow-renneting milks.

Koestler(2, 3) has shown that the coagulation of some slow-renneting soft-curd milks in Switzerland was not improved by the addition of hydrochloric acid. Addition of calcium chloride effected an improvement. Similar experiences have frequently been met with in cheese factories in New Zealand. With normal milks the renneting time is lowered and the firmness of the curd is increased by increase of the ripening period before addition of rennet, but in most cases of trouble with soft-curd milk, the longer ripening period gives no improvement.

In one factory in New Zealand where such trouble was being experienced rennet tests and classifications of curd type were made on all of the 15 individual milk suppliers, (*a*) in the fresh state, (*b*) with addition of 0·2 ml. *M*/2 calcium chloride solution, (*c*) with addition of 0·4 ml. *M*/2 calcium chloride solution, (*d*) with addition of 0·4 ml. *N*/2 hydrochloric acid. The results are given in Table IX. The curd classifications are quoted on the numerical scale to facilitate averaging.

The addition of calcium chloride in both concentrations thus diminished the renneting time, and increased the firmness of the curd. The addition of 0·4 ml. *N*/2 hydrochloric acid, on the other hand, generally decreased the renneting time to one half the original value, but had little effect on the type of coagulation obtained, the average "curd firmness" being raised only from 2·9 to 3·3. All the individual milk supplies (except Nos. 6 and 7) did not respond to the action of hydrochloric acid.

A number of individual cow samples of milk of the slow-renneting soft-curd type have been examined from this point of view and some of them have given results similar to those described. The results for one cow on five successive days, and with graduated additions of both calcium chloride and hydrochloric acid, are given in Table X. The averages are plotted in Fig. 7.

The effect of calcium chloride on renneting time was thus regular and definite, and there was a progressive increase in firmness of the curd. The effect of acid on the other hand was first to increase the renneting time, and then to

decrease the value steadily with increasing additions of acid, but there was little improvement in the type of curd obtained. Some recent work with soft-curd milks from cows at the end of lactation has indicated that many of these are unresponsive to the action of both hydrochloric acid and calcium chloride,

Table IX. *Renneting times and firmness of curd for milks of fifteen suppliers to a cheese factory at a time when coagulation difficulties were being experienced. Effect of addition of calcium chloride and hydrochloric acid on these properties*

	Fresh milk		0·2 ml. *M*/2 $CaCl_2$		0·4 ml. *M*/2 $CaCl_2$		0·4 ml. *N*/2 HCl	
Suppliers no.	Renneting time (sec.)	Curd type	Renneting time (sec.)	Curd type	Renneting time (sec.)	Curd type	Renneting time (sec.)	Curd type
1	44	6	35	6	27	8	30	5
2	46	0	31	5	21	7	25	2
3	42	5	25	6	24	7	24	5
4	46	5	31	6	24	7	28	3
5	40	6	26	5	23	9	22	6
6	77	1	25	3	20	7	20	6
7	49	1	32	6	24	8	26	4
8	34	5	24	4	20	6	20	5
9	69	1	43	2	27	3	35	0
10	48	4	30	3	21	8	23	3
11	43	2	24	3	21	5	20	1
12	39	2	31	3	22	7	27	2
13	55	0	31	6	22	7	28	1
14	53	4	33	6	23	8	28	3
15	63	2	32	5	21	8	32	3
Average	50	2·9	30	4·6	23	7	26	3·3

Table X. *Effect of graduated additions of calcium chloride and of hydrochloric acid to milk of an individual cow over five successive days*

	Renneting time in seconds					
Date	4. v. 31	5. v. 31	6. v. 31	7. v. 31	8. v. 31	Averages
Original milk ...	525	230	270	270	184	296
+0·05 ml. $CaCl_2$	340	115	170	149	153	187
0·10	156	109	136	107	114	124
0·20	92	59	73	73	64	72
0·30	50	45	43	52	47	47
0·40	41	35	36	33	35	36
0·50	32	—	—	—	—	—
0·2 ml. HCl	—	330	477	216	420	361
0·3	—	310	276	177	217	245
0·4	—	270	149	174	167	190
0·5	—	66	92	117	144	105
0·6	49	105	71	54	76	77

so far as the firmness of the curd is concerned, although they give shorter renneting times with these additions. The classification of soft-curd milks from individual cows according to their responsiveness to the action of calcium chloride and hydrochloric acid will be further studied at this Institute during the next dairying season.

Curd tension and heat coagulation.

Experiments with hard- and soft-curd milks on their stability to heat coagulation according to the method of Sommer & Hart(24) showed that there was no connexion between hardness of renneted curd and heat coagulation. Both firm- and soft-curd milks coagulated. These findings are in agreement with those of Welch & Doan(10).

The manufacture of cheese from slow-renneting soft-curd milk.

For 5 days in October 1931, morning's milk was segregated from eight cows giving hard-curd milk and from seven cows giving soft-curd milk, and

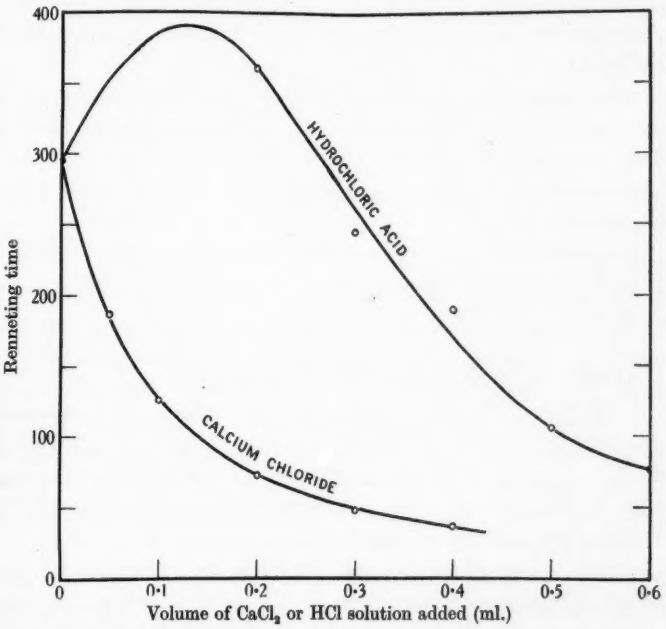

Fig. 7. Average renneting times of slow-renneting milk treated with varying proportions of $M/2$ hydrochloric acid solution and $M/2$ calcium chloride solution.

cheese was made in 20 gallon vats, from the two types of milk. The soft-curd milk reached the "cutting" stage 20 min. later than the hard-curd milk, and the whey was run (or dipped) at an acidity 0·02 lower than that for the hard-curd milk. The acidities at drying were the same.

The composition of the milks and wheys are shown in Table XI.

The following points may be noted:

(1) The fat contents of the wheys were low in all cases, but were virtually equivalent for the two types of milk (see McDowall(23), pt. 2). As the slow-renneting soft-curd milks had lower fat contents the proportion of fat lost in the whey was greater for these milks. The results, however, are quantitatively very different from those recorded by McDowall(23) for soft-curd milk in a

Table XI. *Fat and protein contents of quick- and slow-renneting milks and of the wheys from these milks at the "running" stage of Cheddar cheese making*

Date	12. x. 31		13. x. 31		14. x. 31		15. x. 31	
Milk types	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
Fat in milk	4·9	3·4	4·8	3·6	5·0	3·3	4·8	3·6
Casein in milk	2·78	2·20	2·87	2·40	2·88	2·49	2·92	2·45
Total protein in milk	3·73	3·04	3·65	3·12	3·70	3·09	3·66	3·08
Total protein minus casein in milk	0·95	0·84	0·78	0·72	0·82	0·60	0·76	0·63
Fat in whey (W.S.)	—	—	0·14	0·14	0·20	0·17	0·19	0·19
Protein in whey	1·11	0·86	1·06	0·88	1·03	0·84	0·99	0·85

commercial factory where the softness of curd definitely gave a considerably increased fat loss.

(2) The protein losses bear approximately the same relationship to the non-casein fraction for both types of milk, i.e. the softness of curd has not appreciably accentuated the casein losses.

(3) The results for both fat and protein loss were much lower than those of Hill & Merrill.

(4) The cheese from both types of milk were of first grade quality.

(5) The cheese-making procedure had to be varied to suit the types of milk in the vat.

Effect of temperature on the renneting time and curd type.

Apart from the addition of calcium chloride, or, with some slow-renneting milks, the adoption of a longer ripening period or the addition of mineral acid, the possibilities of improving the renneting qualities of milk for cheese-making appear to be restricted to either (*a*) increase of temperature of setting, or (*b*) increase in the quantity of rennet.

(*a*) *Increase of renneting temperature.* Parisi[25] has concluded that the temperature coefficient of coagulation of milk with rennet is approximately constant for a single milk under varying conditions, but varies from 1·35 to 2·09 for different milks. Caulfield & Riddell[20] found that the curd tension results for the Hill test were largely influenced by the temperature of coagulation. Reference has already been made to the difference in the temperature of setting of milk for Swiss and for Cheddar cheese manufacture. The difficulties described by Koestler[2, 3] refer to the setting of milks at 35° C. (95° F.) which is much higher than the normal setting temperature of milk for Cheddar cheese. The Hill curd test also is carried out at 35° C. It is obvious, therefore, that a raising of the setting temperature will not prove a universal remedy for the renneting trouble in Cheddar cheese making. It is possible, however, that a rise in setting temperature in some cases may mitigate the difficulty. The opportunity has not yet presented itself for testing this possibility on the commercial scale, although the use of setting temperatures as high as 35° F. has been tried (for other reasons) at this Institute, and has been found to be

workable, provided the commencement of cooking is delayed sufficiently after the "cutting" stage.

The authors have made some experiments on the effect of temperature on the coagulation of some slow-renneting milks from individual cows. An instance in which the results were positive is given in Table XII. It will be seen that the rise in temperature improved the coagulation, but even at 38° C. the coagulation was not satisfactory.

Table XII. *Effect of temperature on renneting time and curd type with slow-renneting milks from individual cows*

	Milk		
Temperature °C.	Renneting time (sec.)		Curd type
30	>240	0	No coagulation
32	>240	0	No coagulation
34	180	0	Coagulation
36	104	0	Coagulation
38	86	1	—

(*b*) *Increase in the quantity of rennet.* As already indicated the proportion of rennet used in the beaker test adopted by the authors was greatly in excess of that commonly used in the cheese vat. Under normal commercial conditions, it is the common experience of cheese-makers that the setting time and the type of coagulation can be controlled by varying the proportion of rennet added within the limits of 3 and 5 oz. per 1000 lb. milk (as may be deduced from the law of Storch & Segelcke); and enquiry from factory managers in different districts has shown that there is a variation within these limits in the proportion of rennet normally added (see also Koestler(2, 3)). In the instances where "soft-curd" difficulties have been acute, due to an abnormality in the milk, it has been the common experience in New Zealand that addition of even as much as 9 oz. of rennet per 1000 lb. of milk does not greatly improve the coagulation. The addition of such large quantities of rennet is undesirable from a cheese quality point of view, as it may, and often does, result in development of pastiness of body and bitterness of flavour (compare Riddet *et al.*(26)). The present authors found, with a milk which gave no coagulation in 240 sec. in a rennet test according to their technique, that there was still no coagulation when the proportion of rennet had been increased to eight times the normal proportion used. This milk, treated with 0·4 part by volume of *M*/2 calcium chloride solution, gave a coagulation in 101 sec., and a definite curd which was graded as "soft" on the scale cited earlier.

It appears therefore that in acute cases of slow-renneting trouble, the only recourse is the addition of calcium chloride to the milk in the vat. The quantity to be added should be determined by means of experimental trials on the milk with different quantities of calcium chloride added.

Discussion

The difficulties experienced with the rennet coagulation of milk in cheese factories for the most part is not due to the tendency of some individual cows habitually to give soft-curd milk. Such milk is usually such a small proportion of the total supply that the effect on the coagulation is of only minor importance (see above, Fig. 6). The main trouble is only of spasmodic occurrence, and, it seems, must be related to external conditions. On this side of the question there is but little information available. Hill(6) has formed the tentative conclusion that the curd tension of milk, as measured according to his procedure, is lower in summer when the animals are on pasture than in winter when they are on dry feed, and has observed a decided hardening of the curd during very cold weather. Other workers have claimed that there is no seasonal variation in the curd tension of milk. As far as the renneting of milk under cheese-making conditions is concerned there appears to be no doubt that slow-renneting soft-curd milk is more prevalent during periods of dry weather. This subject has already been discussed by one of us(23), and it has been shown from examinations of both fat losses and fat recoveries from whey that during dry periods there is a general increase in fat loss coincident with soft-curd conditions in the milk. Apart from the effect of drought it appears also that some districts are more liable to encounter renneting difficulties than are others. Lundstedt(18) states that during an epidemic of foot and mouth disease in Denmark in 1924, he had difficulty in obtaining a firm curd for cheese-making, and expresses the opinion that the curd type is determined by the character of the casein complex. The finding of Cherbuliez and his co-workers and of others (see (23)) that casein is heterogeneous and that one of its main components is less easily coagulated by rennet than the other, lends support to Lundstedt's opinion. On the other hand the cause of softness of curd and slow coagulation may be related to the state of combination of citric acid in milk, since, as is well known, sodium citrate can prevent the curdling of milk by rennet (Bosworth & Van Slyke(27)).

The problem thus is twofold: (*a*) What is the cause of the production at *certain times* by the cows in a herd or in a district of milk which coagulates slowly and gives soft curds? (*b*) What are the differences in the composition or constitution of the normal milk of these herds, and the slow-renneting milk which they produce spasmodically?

Some clarification is also required of the relationship of persistently soft-curd milks from individual cows to the slow-renneting soft-curd milks produced spasmodically and simultaneously in a district by a number of herds of cows which, under most conditions, give normal milk.

Summary

Apart from mastitis milk there appear to be two types of soft-curd milks:

(1) Milks of individual cows which throughout a lactation or throughout their lives persistently give soft-curd milks. Such milks readily respond to additions of acid and appear to be due to the breed and individuality of the cow and not to any abnormality.

(2) Milks of cows or herds which, though normally giving a satisfactory coagulum, suddenly become rennet-resistant for a certain period. Such milks are more difficult to deal with as in general they do not respond to additions of acid unless these additions are large.

There is little evidence available to show whether the two types are regularly related, but some individual cows in a herd do produce soft curd milk of the type which is unresponsive to the addition of acid regularly throughout a season when the majority of the cows in the herd are giving normal renneting milk. In the work described above on individual cow's milk there has been no attempt to classify the milks according to the effect of acid on the coagulation, although it is now realized that such a course would have been desirable.

Soft-curd milks are more commonly produced by some breeds of cows than by others. On the average Ayrshire cows give milk with longer renneting times than Friesian cows, and Friesian cows give milk with longer renneting times than Jersey cows. The renneting time of milk from Ayrshire cows shows a steady and marked increase throughout the period of lactation. The same increase is noticeable with Friesian and Jersey milk, but it is much less distinct. The firmness of the rennet curd, classified on an arbitrary scale, shows on the average little variation throughout the period of lactation, Friesian and Ayrshire milk curds being softer than Jersey milk curds. With Ayrshire and Friesian milk the curd firmness increases with increasing fat content of the milk, but there is little variation for Jersey milk. For milks of all three breeds soft curds are encountered most frequently in milks which coagulate slowly.

Dilution of normal milk with 15 per cent of water does not seriously affect the coagulation. Concentration of normal soft-curd milk under reduced pressure at a low temperature results in more rapid coagulation and a firmer curd.

Slow-renneting soft-curd milks from individual cows, mixed with hard-curd milks, in all cases examined, showed a greater lowering of renneting time and a greater hardening of the curd than the properties of the admixture would indicate if it be assumed that the properties were additive.

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150. THE EFFECT OF CERTAIN METALLIC CONTAMINANTS ON THE CHEDDAR CHEESE MAKING PROCESS

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Many investigations have been carried out on the defects in quality produced by the presence of traces of metallic contaminants in butter. In the case of cheese, however (particularly Cheddar cheese), the effects of metallic contamination on the quality do not appear to have been studied, except from the medico-legal aspect of food-poisoning.

There are references to localized stains and discolorations in cheese, which have been ascribed to the presence of certain metals found to be concentrated in these areas. Leitch (1) found that the presence of a few parts per million of lead in milk used for cheese-making caused muddy spots to develop throughout the cheese, and that salts of zinc, iron and copper had no effects on the colour. Davies (2) found traces of tin (derived from tin solder) in darkened areas of Cheddar cheese, but considered the lead constituent of the solder to be responsible for the colour defect. Orla-Jensen (3) noted that both iron and copper promote dark discoloration in cheese, and considered the discoloration caused by iron to be due to the presence of ferrous salts, which undergo oxidation (by the air) to a colourless form. With copper, he considered the reverse reactions to occur. These three observers all consider the types of discoloration noted to be dependent upon the presence of sulphur compounds, possibly hydrogen sulphide in the cheese.

The investigations reported in this paper were made mainly in order to study the effects of minute amounts of metal added in a soluble form to the milk. The amounts added were considered to be of the order of those expected in a fairly heavily contaminated milk supply (about 3 p.p.m. of metal). At the same time cheeses were made from milks containing 7 p.p.m. of metal, which is an extremely high degree of contamination unlikely to be encountered in practice, but of interest from the experimental point of view.

The metals were added just prior to the addition of the starter (simulating "factory contamination"). In practice, contamination may occur in the milking shed as well as in the factory, and (in the case of the copper and iron) the experiments were repeated in order to simulate the condition of "shed contamination". In these latter experiments, one-half of the addition of metal salts was made at night and the remainder on the following day just after pasteurization of the milk.

The six metals studied in this investigation were chosen for the following reasons: Iron and copper are extensively used in dairy appliances and machinery, and these metals are invariably present in traces in dairy products.

Manganese is used in certain alloys, zinc for galvanizing, lead in solder and paints, while aluminium and its alloys are becoming increasingly important in the fabrication of dairy factory plant.

In some parts of New Zealand the water supply contains relatively high amounts of iron salts, and the milk which comes from sheds supplied with this water (used for washing the milking machines and utensils) often contains traces of iron. The quality of the cheese made by certain factories in these districts is not as good as the management and hygienic conditions of the factories would appear to warrant, and the ferruginous nature of the local water supply has been suspected as being responsible for the off-flavours frequently noted in cheese from these districts.

Experimental

Manufacture of cheese

Each day's supply of mixed milk was flash-pasteurized at 153° F., and was apportioned in three vats. To two of the vats the metallic salt (sulphate or chloride), dissolved in a small amount of water, was added, the concentrations in the milk being approximately 3 and 7 p.p.m. respectively of the metal under examination. The third vat was used for the control cheeses.

Manufacturing and curing conditions of the cheeses were uniform for each set of comparisons. All the cheeses manufactured were coloured with annatto. Two cheeses were made from each vat. It is considered that the major differences in cheese quality noted can be ascribed to the metallic contamination of the original milk.

Examination of cheese

One of each pair of cheeses was examined at 14 days after manufacture, the plughole being subsequently filled with wax. The other cheese was tinned under reduced air pressure at about the same time. The tinning of the cheese was designed to reduce the entry of air through the slits into the cheese in an attempt to differentiate between (1) the defects caused by the metals on the general process of ripening, and (2) the defects caused by the catalytic effects of the metals on the oxidation processes initiated by the atmospheric oxygen entering from the outside of the cheese. These cheeses were cut and examined at 9 months after manufacture and were thus considerably older than is usually the case for average commercial cheeses when examined. They had been kept at about 60° F. which is higher than commercial storage temperatures. The cheeses were examined mainly from the point of view of discoloration defects. The cut cheeses were examined several days later, and were then waxed to prevent excessive drying out, and were recut for final examination after some weeks' storage at room temperature.

Analysis of milk and cheese

The estimations of the metals at various stages of the manufacturing process were made on solutions of the inorganic constituents of the cheese from which the organic matter had been removed by the process of wet-ashing.

Pyrex ware, specially reserved for this work, was used throughout, and blank determinations were run with each set of analyses. The analytical processes employed were: copper—sodium diethyl dithiocarbamate (4), iron—ammonium thiocyanate (5), lead—diphenylthiocarbazone (6), manganese—persulphate (7). Zinc and aluminium were not estimated.

The distribution of metallic contaminants during the Cheddar cheese making process

The results are given in Table I.

Table I. *Distribution of metallic contaminants during the Cheddar cheese making process. Results expressed in parts per million*

The whey was filtered before analysis in every case

Column	1	2	3	4	5	6
Metal added	Iron*					
Proportion added (p.p.m.)	3·0	7·0	3·0	7·0	5·0†	20·0†
Conditions	Just before starter		½ overnight ½ prior to starter		Just before starter	
Metal found:						
In milk	3·3	6·3	3·7	10	5·0	22·2
In whey at approx. 1-hour cooking	—	2·2	—	6·5	—	—
In run whey	1·4	2·6	2·0	4·7	1·2	8·1
In whey at approx. 1-hour cheddaring	—	5·0	—	6·8	—	—
In press—whey	—	11·8	—	13·1	—	—
Retained in cheese ‡	20	50	24	61	27	133
Ratio of total metal found in cheese to total metal added to milk §	0·65	0·7	0·8	0·85	0·55	0·65

Column	7	8	9	10	11	12
Metal added	Copper					
Proportion added (p.p.m.)	3·0‖	7·0‖	3·0	7·0	3·0	7·0
Conditions	Just before starter		Just before starter		½ overnight ½ prior to starter	
Metal found:						
In milk	—	—	2·7	7·4	3·0	9·0
In whey at approx. 1-hour cooking	—	—	—	1·2	—	1·2
In run whey	0·5	2·7	0·1	1·4	0·7	1·5
In whey at approx. 1-hour cheddaring	—	—	—	3·1	—	3·8
In press—whey	—	—	—	5·6	—	5·4
Retained in cheese ‡	—	—	31	78	—	—
Ratio of total metal found in cheese to total metal added to milk §	—	—	1·0	1·1	—	—

Column	13	14	15	16	17	18
Metal added	Copper		Lead		Manganese	
Proportion added (p.p.m.)	5·0†	20·0†	3·0	7·0	3·0	7·0
Conditions	Just before starter		Just before starter		Just before starter	
Metal found:						
In milk	4·9	20·7	4·7	—	—	—
In whey at approx. 1-hour cooking	—	—	—	—	—	—
In run whey	Nil	0·5	<1	<1	<1	<1
In whey at approx. 1-hour cheddaring	—	—	—	—	—	—
In press—whey	—	—	—	—	—	—
Retained in cheese ‡	52	198	22	28	20	34
Ratio of total metal found in cheese to total metal added to milk §	1·0	1·0	0·7	0·4	0·7	0·5

* Added as the ferrous salt. † Preliminary experiments.

‡ Calculated to 30 per cent moisture basis. § Assuming 10 lb. milk has made 1 lb. of cheese.

‖ Added in the form of rust.

The results show that, in the case of copper, nearly all of the metal was retained in the curd. Iron disappeared to a greater extent in the whey, leaving about two-thirds of the amount originally added concentrated in the final cheese. The marked rise in concentration of the metal in the whey as the cheese-making proceeds, which is governed by the increasing acidity, has also been observed by Davies(8). In the case of lead and manganese, more than one-half of the metal originally added was retained in the curd.

When the iron salt was added partly overnight and partly just prior to the addition of "starter" on the following day, the proportions retained in the cheese were rather higher than when the metal was all added just before manufacture. Iron added as rust was partly converted to a soluble form and a proportion was retained in the curd. (Even iron and copper filings were gradually dissolved by the acids of the cheese.)

The effect of metallic contamination on cheese quality

(*a*) *Body and texture.* When plugs were examined at about 14 days after manufacture, there was evident a tendency for the metalled cheeses to be "sweet", i.e. not to have attained the normal degree of ripening expected at this stage. This defect was not marked, nor was it quite consistent, as the cheeses containing 3 p.p.m. of metal were sometimes more "sweet" than those with 7 parts. When the cheeses were finally cut at 9 months (or older), no significant differences in body and texture were noted. The body in all cases was rather poor owing to the high loss of water which is inevitable in small cheeses when stored for long periods.

(*b*) *Flavour.* The flavours of all the cheeses at 14 days after manufacture were normal. When the cheeses were finally examined, after storage for 9 months or more, the flavours varied greatly. The cheeses stored in tins were quite different in nature from ordinary New Zealand Cheddar cheese, but the gradings for these confirm the findings for the normally cured products.

The grading points allotted for flavour to cheeses containing 3 and 7 p.p.m. of copper, iron, aluminium and lead were about $1\frac{1}{2}$–$2\frac{1}{2}$ points respectively lower than for the control cheese, the "metalled" cheeses being bitter, sharp and pungent. These defects were less pronounced for the cheeses containing manganese, and were not observed in the cheeses containing iron-rust, or iron added in two stages, i.e. partly overnight and partly just prior to manufacture.

Metallic contaminants have possibly three main effects on the cheese-ripening process: (i) on the chemical changes occurring in the process of protein degradation, (ii) on the action of the rennet enzymes, and (iii) on the growth of the bacteria responsible for acidity development and flavour production.

(i) Davies *et al.*(9) have observed that the addition of 36 p.p.m. of copper to Cheddar cheese appreciably inhibited the protein degradation, especially the formation of amino acids.

(ii) Traces of metals have been found to alter the rate of reaction of many enzymes, and Loevenhart(10) and Lorcher(11) have shown that the rennet

coagulation of milk is affected by various metal ions. The cheese-making records for the vats containing metallic contaminants (as described earlier) showed no significant differences for their setting times, but the concentrations of the metals were much lower than those studied by the two authors just quoted. However, as the metals are concentrated in the curd during the process of manufacture, it is possible that they may then affect the activity of the rennet enzymes in the subsequent ripening process.

(iii) Although the lactobacilli play the most important part in the development of cheese flavours, the early stages, in which the streptococci predominate, are also critical in determining the quality of the product. The effect of certain metals upon the acidity production by a commercial "starter" (containing mixed strains of lactic streptococci) when grown in pasteurized whole milk was examined. When added at the rates of 2·5 and 7·5 p.p.m., Mn″, Ni″, Pb and Zn had no effects. Cu″, Fe″ and Fe‴ accelerated the development of acid, but in the case of copper the effect changed to one of inhibition when the clotting stage was approached.

The action of metals on acidity production in renneted milk was followed by the vitality test of Whitehead & Cox (12). An inhibition of acidity production was noted only in the case of copper, which might be expected from its behaviour in clotted milk. In the actual process of manufacture the inhibitory effect of copper on the acid production was very marked.

(*c*) *Colour.* No pronounced differences between the colour of plugs taken from "metalled" and control cheeses were noted at about 14 days after manufacture. When the cheeses were finally examined various marked defects in colour were observed.

(i) *Tinned cheeses.* The colours were all similar to those of the controls, except in the case of the cheeses containing copper, which were slightly darker in colour, and those containing lead which were spotted.

(ii) *Normally cured cheeses.* Cheeses containing iron-rust, aluminium and zinc were similar to the control cheeses. The cheeses with lead contamination had numerous dark spots, but were no worse than those which had been tinned. All of the cheeses made from milk contaminated with copper were darker than their control cheeses, while the cheeses containing iron salts showed wide bleached and "muddy" zones ($\frac{1}{4}$–$\frac{1}{2}$ in. wide) at many of the slits.

When recut after keeping for several days the cheeses from both the tinned and untinned series containing iron-rust showed "muddy" zones at the slits, and the "copper cheeses" had even darker zones at the slits than the "iron cheeses" had originally shown.

After a further storage of the cheese (waxed) for several weeks, the original "muddy" defects noted for the "copper and iron cheeses" were very much more pronounced. In this respect the tinned cheeses were even more discoloured than the ordinary ones, although they had, in all, only about one-fourth as long exposure to the air as those cured without tinning.

It is noteworthy that the discoloration of cheeses containing traces of

copper and iron is conditional upon the access of air to the interior of the cheese through the slits.

The role of hydrogen sulphide in the discoloration of cheese by metallic contamination

As other workers have concluded that the metallic sulphides are responsible for the discoloration observed in cheeses which have been contaminated by metals, most of the cheeses referred to in this paper were examined immediately after cutting by the nitroprusside test, which, when applied in the following way, is sensitive to about 2 p.p.m. of sulphydryl (added as cystein): 1 g. of cheese was ground for about 15 sec. with a little water and 0·2 g. of ammonium sulphate containing 2·5 per cent of sodium nitroprusside,[1] and 10 drops of strong ammonia solution were then added. The relative intensities of the colours were noted. The results given in Table II refer to portions of the cheese selected from undiscoloured areas, remote from edges, slits, or cracks. Except in the case of the lead series a sulphydryl reaction was not given by any discoloured portion of a cheese.

Table II. *Sulphydryl reaction of undiscoloured interior portions of metal-contaminated cheeses*

Ref. no.	Date of manufacture	Nature of experimental cheeses and method of curing	Nitroprusside reaction for sulphydryl
1	24. xi. 33	Control (air)	Very deep
1*a*		Control (tinned under reduced air pressure)	Deeper than 1
2		From milk 3 p.p.m. copper (air)	No reaction
3		„ 7 „ „ (air)	No reaction
3*a*		„ 7 „ „ (tinned)	No reaction
4	27. xi. 33	Control (air)	Deep
5		From milk 3 p.p.m. iron (air)	Fairly deep
6		„ 7 „ „ (air)	Fairly deep
7	28. xi. 33	Control (air)	Deep
8		From milk 3 p.p.m. lead (air)	Deep
9		„ 7 „ „ (air)	Deep
10	29. xi. 33	Control (air)	Deep
11		From milk 3 p.p.m. zinc (air)	Fairly deep
12		„ 7 „ „ (air)	Slight
13	30. xi. 33	Control (air)	Deep
14		From milk 3 p.p.m. aluminium (air)	Deep
15		„ 7 „ „ (air)	Deep
24	4. xii. 33	From milk 7 p.p.m. iron (air)	Slight

From the foregoing table it is seen that Cheddar cheese develops an appreciable amount of hydrogen sulphide, or more strictly sulphydryl during the ripening process. Only in the case of copper, and to a less extent of iron and zinc, did the presence of the metal retard the reduction of the protein sulphur to sulphydryl. The combination of the sulphydryl ion with the metal it is considered would not remove any notable amounts of free sulphydryl, because in

[1] The reagent must be well ground, and it is advisable to prepare it freshly each day.

the lead-contaminated cheeses where the discoloration is undoubtedly caused by the formation of lead sulphide, the intensity of the nitroprusside test was not reduced. The absence of sulphydryl in the "copper"- and its diminution in the iron- and zinc-contaminated cheeses is probably due to the effect of these metals in retarding normal production of sulphydryl substances by the lactobacilli in the ripening process. Davies *et al.* (9) have shown that copper when added at the rate of 36 p.p.m. to cheese curd "hindered the attainment of the normal reducing intensity (i.e. raised the oxidation potential), and appreciably inhibited the breaking-down of the proteins, particularly the formation of amino acids". It has subsequently been found that sulphydryl is present in all mature New Zealand Cheddar cheese, and is therefore an end-product in the protein degradation.

Further proof that the "muddy" discoloration of cheeses containing iron and copper was not caused by the formation of sulphides was obtained by grinding small portions of mature cheese (from which the sulphydryl had been removed by exposing the shredded cheese to air overnight) with solutions giving concentrations of 20 and 50 p.p.m. of ferric iron, and 30 and 70 p.p.m. of copper. Under these conditions, the "metalled" cheese mixtures darkened noticeably when compared with the control. When portions of this cheese were ground with lead (50 p.p.m.) no coloration was apparent, although a distinct yellow-brown colour was obtained with fresh cheese in which sulphydryl was present.

The rapidity with which the discoloration develops in (initially) normally coloured portions of Cheddar cheese contaminated with copper and iron when exposed to air suggests that the reaction is an oxidation of a preformed metal : protein complex, which has been present in the reduced colourless form. It is not considered that the change is simply the formation of "melanin" from tyrosine (this amino acid is present in mature New Zealand Cheddar cheese), since Raper (13) has shown that tyrosine even when oxidized with Fenton's reagent (hydrogen peroxide and ferrous iron) takes some hours to develop a dark colour. The actual nature of this chemical change in cheese is therefore still obscure.

SUMMARY

Traces of certain metallic contaminants (copper, iron, lead, zinc, aluminium and manganese), when added at the rate of 3 and 7 p.p.m. to milk used for the manufacture of Cheddar cheese, cause certain flavour (and colour) defects in the matured cheeses, of which, however, no indication is given when the cheese is plugged and graded at about 14 days after manufacture.

The distribution of copper, iron, manganese and lead between curd and whey during the cheese-making process is recorded and the action of traces of various metals on the acidity development in a typical "starter" culture has been followed.

The discolorations noted for copper- and iron-contaminated cheeses are considered to be due not to the formation of their sulphides (although this is the case for lead discoloration), but to the atmospheric oxidation of a colourless metal : protein complex.

ACKNOWLEDGEMENTS

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151. THE REACTIONS AND PROPERTIES OF ANNATTO AS A CHEESE COLOUR

WITH PARTICULAR REFERENCE TO THE CHEMISTRY OF CHEESE DISCOLORATION

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(With 3 Figures)

The commercial cheese colour known as annatto consists essentially of a strongly alkaline aqueous extract of the tropical seed *Bixa orellana*, and is a complex product containing approximately 10 per cent of solids, of which only a minor proportion is the actual colouring agent.

According to recent work, notably by Karrer and his associates, it appears that the colouring principle in the annatto seed is present as bixin, to which Kuhn & Winterstein (1) have given the following constitution:

$$HO_2C(CH : CH.CMe : CH)_2CH : CH(CH : CMe.Ch : CH)_2CO_2Me.$$

The potassium salt of norbixin (the free dicarboxylic acid derived from bixin) is believed to be the tinctorial agent present in commercial annatto solutions used as cheese colours.

The properties of commercial annatto solutions have not, so far as we are aware, been examined from the point of view of cheese-making as the colour plays no essential part in this process. It was only through an outbreak of discoloration occurring in coloured New Zealand cheese made in the 1930–1 season that the attention of this Institute was drawn to this subject.

Experimental

(i) *General properties and reactions.* The effects on the colour of initial concentration in the milk, acidity (*p*H) of the medium, distribution during manufacture, etc., have been studied.

(ii) *Biochemical changes.* As the changes associated with the cheese-ripening process are of a biochemical nature, the action of several enzymes on annatto has been examined.

(iii) *Chemical changes.* The types of discoloration ("bleached", "pink" and "muddy") prevalent in the outbreak during the 1930–1 season were associated with the slits and cracks of the affected cheeses, as pointed out by Morgan (2) and Moir (3). These fissures, on dissection of the cheese, could always be traced, often by a moist sinuous path, to cracks in the rind. It was therefore considered that the defect was connected with the entry of air (oxygen) into

the cheese, and the oxidation and reduction of annatto solutions has therefore been studied in some detail.

Except where otherwise indicated, the tests described in this paper have been carried out on solutions of fresh commercial annatto (in use at this Institute's factory), diluted to 1 : 10,000, which is of the order of dilution used in milk for Cheddar cheese making. The examinations for colour strength have been made in a B.D.H. pattern Lovibond tintometer, fitted with universal light attachment, using a 1 cm. cell. Unfortunately, the colour of annatto solutions (yellow, with a small proportion of red) is not easy to assess, and is usually "overbright" necessitating the use of a neutral tint. Colours beyond

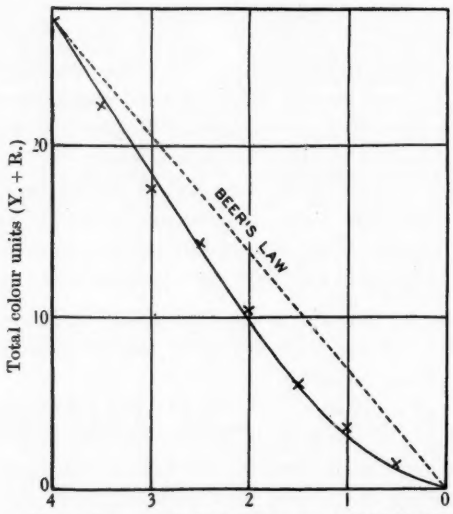

Fig. 1. The effect of dilution on annatto solutions.

the range of 1·5–10 yellow are probably subject to fairly high observational errors, and the amount of neutral tint added may affect rather seriously the matching of the red units. In accordance with the practice of various workers for the tintometric estimation of the related substance carotene, the red (for some series of observations) has been neglected, the colour being returned in yellow units only.

General reactions and properties of annatto

(i) *Effect of dilution* (Fig. 1). The intensity of colour (in transmitted light) is not quite proportional to the dilution, i.e. annatto solutions do not follow Beer's law strictly, but the divergence over the range of strengths of colour added in commercial cheese-making practice would not be of any significance.

(ii) *Effect of reaction* (*p*H) *of the medium.* The change on acidifying a solution of annatto is apparently as follows:

Alkaline	Acid (*p*H 5·5 as in average cheeses)
Potassium salt of norbixin (soluble)	Free acid (norbixin) partly precipitated
Yellow with a little red	Reddish tint (colloidal solution of about $\frac{1}{2}$ tinctorial power of soluble form)

The change in colour in relation to *p*H is seen more clearly when the annatto is examined by reflected light on a solid medium. For this purpose, filter papers previously soaked in annatto solutions were immersed in appropriate buffer solutions and covered with liquid paraffin to prevent evaporation of the liquid. The results are recorded in Table I.

Table I

*p*H ...	6·0	5·7	5·4	5·1	4·8	4·5
Y.	14·1	8·4	8·3	6·2	6·1	5·4
R.	8·3	11·0	11·3	11·6	13·0	13·0
Total colour units	22·4	19·4	19·6	17·8	19·1	18·4
Red/total colour (%)	37	57	58	65	68	71

(The percentage red/total colour is higher than would be found in practice because of the presence of the yellow pigment carotene in the fat of the cheese.)

It has been observed that cheeses of high acidity tend to be deeper in colour than the average.

(iii) *Distribution of annatto in the cheese-making process.* In this experiment, four vats of cheese were made on 1 day from portions of the same batch of milk under uniform conditions of manufacture, the colour ("double strength") being added to the vats in the following proportions: (1) control none, (2) half usual colour 1 : 26,000, (3) usual colour 1 : 13,300, and (4) double usual colour 1 : 6540.

A solution of the annatto (in $N/10$ soda) at the same dilution as used for vat (3) (i.e. the normal amount) had the following colour: 4·7 Y., 0·4 R., 0·1 N.T.

The wheys at running had the following tints (18 mm. depth by reflected light):

Table II

Vat	Colour			Advance in total colour over control units
	Y.	R.	N.T.	
(1) Uncoloured	1·6	0·2	0·1	—
(2) Half usual colour	2·5	0·3	0·1	1·0
(3) Usual colour	3·3	0·3	0·2	1·8
(4) Double usual colour	4·4	0·6	0·2	3·2

The colour retained by the wheys was roughly proportional to the amount of colour added, but in order to compare their annatto contents with the amounts originally added it was necessary to obtain the colouring matter in clear solutions. This was achieved by carefully neutralizing the wheys, and

leaving them with ethyl alcohol (to give 80 per cent concentration) for 2 days in the dark. The colouring matter in the filtrates was estimated by matching the extract in Nessler tubes against a control (80 per cent alcohol) to which dilute annatto (1 : 10,000) was added. A correction was made for the natural colour of the whey from the control vat. The proportions of colour originally added which were recovered in the wheys were: vats (2) 11 per cent, (3) 9 per cent, (4) 9 per cent, i.e. approximately one-tenth. Other considerations (e.g. experiments with skim-milk cheeses) indicate that the annatto combines mainly with the protein of milk, and it would therefore appear that the distribution of the colour in the cheese-making process is an adsorption in which the ratios

$$\frac{(C_1)\text{ Colour retained by the whey protein}}{(C_2)\text{ Colour retained by the curd protein}} = 0{\cdot}1\ (K).$$

The cheeses were examined at approximately 1 and 6 months after manufacture, with the following results.

Table III

Cheese	1 month			Moisture %	6 months			Moisture %	Advance in colour over control (1 month)	
	Y.	R.	N.T.		Y.	R.	N.T.		Y.	R.
Vat (1)	3·0	1·0	0·1	35·5	3·5	1·0	0·4	32·0	—	—
„ (2)	4·5	1·6	0·1	34·0	5·3	3·2	0·6	29·0	1·5	0·6
„ (3)	8·5	2·4	0·2	36·0	8·3	4·5	0·6	34·0	5·5	1·4
„ (4)	9·0	4·1	0·2	35·0	10·0	5·3	0·6	30·0	6·0	3·1

There is thus a proportionality between the colour of the cheese and the initial concentration of annatto in the milk. At 6 months, changes due to drying-out (and possibly other causes) had deepened the colours (particularly the red) of the cheeses considerably.

(iv) *The extraction of annatto from cheese.* In order to compare the relative amounts present in normal and discoloured areas of cheese, it was necessary to extract the colouring matter. The following method of extraction has proved to be satisfactory, leaving only a faint colour in the residual curd which is of about the same yellowish tint as commercial lactic casein and from which only negligible amounts of colour can be extracted. Cheese (2 g.) is ground with methyl alcohol (usually 10 ml.) so that, with the moisture of the cheese, the solvent is equivalent to approximately 94 per cent methyl alcohol(4). The mixture is centrifuged, the supernatant liquid is decanted and filtered and the process is repeated with two more lots of 94 per cent methyl alcohol, the combined filtrates being made up to 25 ml. with the 94 per cent alcohol for colorimetric comparison. "White" cheeses, or butterfat, treated in this way do not impart any of their pigments to the methyl alcohol solution.

Morgan(2) and Moir(3), reporting on the outbreak of discoloration in New Zealand cheese during the 1930–1 season, ascribe the "bleached" type (which was the most common) to the action of enzymes in the presence of the air entering through the fissures. These writers considered the enzymes to be

derived from moulds present in the outer regions of the slits, and reasoned that these enzymes had travelled along the slits to the defective areas. Actually, it can be shown that the annatto pigments have been lost in the rind portion of the slit by comparing the extracted pigments on an equal-moisture basis; desiccation in the outer portions of the cheeses causes a deepening of colour which usually masks the defect within 1–2½ in. of the edge. The observation of Moir (3) that "oxygen of the air apparently restores the colour" of bleached cheeses when cut and exposed to the air is considered to be explained by the darkening caused by desiccation.

Biochemical changes

In view of the important part played by enzymes in the process of cheese-ripening, the action of certain enzymes on annatto was examined:

(i) *Catalase.* Leucocytes (from centrifuged milk), washed with normal saline, were suspended in buffer solutions (*p*H 4·2–5·5), together with annatto and hydrogen peroxide. Controls were also run in which the leucocytes had been killed by heat. Catalase was found to have no action on the annatto. The traces of catalase detected in discoloured areas of cheese were not consistently higher than those found in normal portions.

(ii) *Peroxidases and oxidases* (vegetable). An extract containing these enzymes was examined on similar lines to the catalase but no action on the annatto was observed.

(iii) *Dehydrogenases.* As the pigments of annatto are highly unsaturated compounds in which the conjugated double bonds act as chromophores, the possibility of "bleaching" being caused by reduction of the ethenoid linkages was considered. A highly reducing potential is set up in Cheddar cheese during the course of ripening (5) and the possibility of the annatto pigments acting as hydrogen acceptors (6) in reducing actions promoted by bacteria was accordingly examined. The technique of Stephenson (7) was adopted, suspensions of *B. coli* being used. A series of tubes each containing 1 ml. *N*/20 sodium lactate (hydrogen donator) and 1 ml. 0·5 per cent annatto solution, together with various other control solutions, were buffered to *p*H 7·6 or 5·5 (simulating conditions in cheese) and were sealed under high vacuum. After 2 days at 37° C., no diminution in the intensity of the annatto colour was observed in any of the tubes. No evidence of the bacterial reduction of annatto (as referred to by Orla-Jensen (8)) has ever been reported in New Zealand Cheddar cheese, and it would appear that discoloration in coloured cheeses is not likely to be caused by the reducing actions of the organisms present.

Chemical changes

The chemical constitution of bixin and its derivatives indicates the susceptibility of these compounds to both oxidation and reduction. The following observations on the relative importance of these two reactions (when

considered from the point of view of the cheese-making process) have been made:

(*a*) Annatto solutions are scarcely affected by reducing agents (SO_2 and H_2S) if protected from the air.

(*b*) Annatto solutions are bleached by dilute hydrogen peroxide, and slowly by the air (oxygen). The action of air is much more rapid if the solutions are kept in the light.

(*c*) Annatto solutions can replace milk-fat in the oxidation-reduction change promoted by the incidence of sunlight on milk, as recorded by Whitehead (9). The reaction between annatto and methylene blue occurs even in aqueous solutions, as shown by the following results: Tubes containing (*a*) dilute methylene blue solution, (*b*) dilute annatto solution, and (*c*) dilute methylene blue and annatto solutions mixed, were exposed for 2 hours to bright sunlight. The colours were then compared with controls which had been kept in the dark. The methylene blue (*a*) and the annatto (*b*) solutions had each lost approximately one-fifth of their original colours. In tube (*c*), seven-tenths of the methylene blue had disappeared, while the annatto pigments had entirely bleached.

Under these conditions in which an oxidation-reduction reaction analogous with biological oxidation-reduction changes has occurred, the annatto has apparently acted as an oxygen acceptor, according to the reaction:

(methylene blue + 2H)	+	(O + annatto)	→	reduced methylene blue	+	oxidized annatto
Blue		Yellow		Colourless		Colourless

(*d*) Discoloration has never been observed in New Zealand Cheddar cheese protected from the air by tinning, and is, in fact, always associated with the slits and cracks of the defective cheeses.

(*e*) It has been found that the carefully extracted fat, obtained from cheese around the slits leading to cracks in the rind, is usually more oxidized than fat extracted from other portions of the cheese. When a proportion of the fat in milk was replaced by "tallowy" (oxidized) butterfat by means of an emulsifier, the colour of the resulting cheeses was markedly bleached.

It was therefore concluded that oxidation is a considerably more important potential factor in causing discoloration in coloured cheeses than reduction.

Experimental

(i) *The bleaching of dilute annatto solutions by atmospheric oxidation.* In order to determine the effects of air under conditions allied to those pertaining in the commercial storage of cheese, dilute solutions of annatto (1 : 1000, i.e. approximately the concentration present in a coloured cheese) were kept for 6 weeks. The annatto was diluted either with freshly boiled water,[1] or with buffer solution (of *p*H 5·5, simulating mature cheese), and were kept in the dark at approximately 35 or 60° F. until examined. In each series duplicate

[1] These solutions would be slightly alkaline.

tests were made. In one, the annatto solution was covered with a thick layer of liquid paraffin in order to retard the ingress of air, and in the other, the annatto solution was in a thin layer which ensured maximum aeration.

For the tintometric examination the solutions were neutralized and were then diluted to 1 : 10,000 with $N/10$ sodium hydroxide. The results are given in Table IV.

Table IV. *Original colour of annatto solution* (1 : 10,000)
5·1 Y., 0·7 R., 0·1 N.T.

	Samples kept at 35° F.			Samples kept at 60° F.		
Nature of solution	Y.	R.	N.T.	Y.	R.	N.T.
(i) Unbuffered (slightly alkaline):						
(*a*) Protected from the air	3·3	0·6	0·1	2·6	0·6	0·1
(*b*) In contact with the air	0·6	0·5	0·1	0·5	0·6	0·1
(ii) Buffered to *p*H 5·5:						
(*a*) Protected from the air	2·9	0·6	0·1	2·7	0·7	0·1
(*b*) In contact with the air	1·8	0·3	0·1	1·4	0·3	0·1

Observations recorded elsewhere in this paper are confirmed by these results, e.g. the loss of tinctorial power of annatto solutions on keeping, the increased rate of bleaching at the higher *p*H values and the tendency for the yellow component to disappear before the destruction of the red. The main interest lies, however, in the bleaching effect of atmospheric oxygen, which has, in the case of the solutions buffered to *p*H 5·5, reduced the colour of those kept in contact with the air to 60 per cent (at 35° F.) and to 50 per cent (at 60° F.) of the colour in the controls.

It is contended that normal portions of a cheese would discolour if kept in contact with the air for a sufficient length of time, providing precautions were taken to prevent the darkening of the cheese by desiccation, which otherwise would mask the appearance of discoloration.

(ii) *The relative rates of oxidation of annatto solutions.* The colour slowly disappears from annatto solutions, even when stored in the dark. Oxidation tests, using hydrogen peroxide as an accelerator, were made on dilute solutions of annatto (*a*) unbuffered, and (*b*) buffered to *p*H 5·5. The effect of 2 or 5 p.p.m. of copper and iron on the rate of oxidation was also studied. The results are shown in Figs. 2 and 3.

It will be seen that copper markedly accelerates the rate of bleaching of the colour in both solutions, while iron accelerates the change only in the unbuffered (i.e. slightly alkaline) series. It is therefore considered that amounts of iron and copper permitted as impurities in commercial annatto extracts should be as low as possible.

(iii) *The properties of old (oxidized) annatto solutions.* All commercial samples of annatto so far examined have contained a certain amount of insoluble residue. From such solutions, particularly when stored in the light,

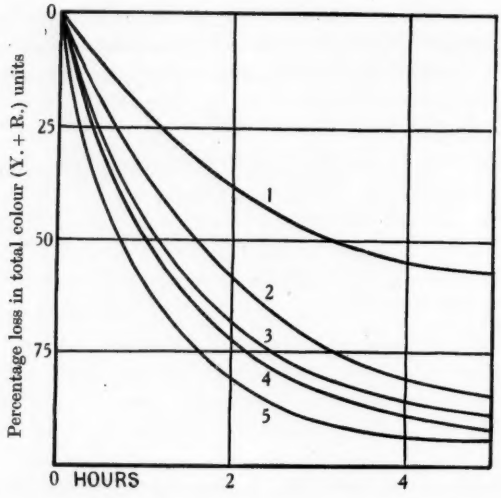

Fig. 2. Oxidation of annatto solutions by hydrogen peroxide. Unbuffered. 1, control; 2, 2 p.p.m. Fe″; 3, 5 p.p.m. Fe″; 4, 2 p.p.m. Cu″; 5, 5 p.p.m. Cu″.

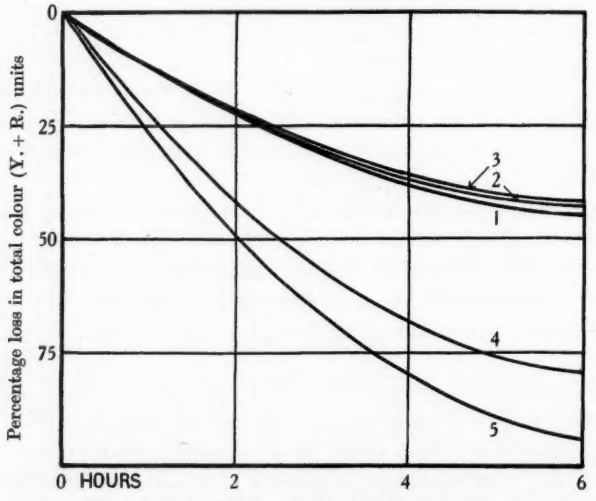

Fig. 3. Oxidation of annatto solutions by hydrogen peroxide. Buffered to *p*H 5·5. 1, control; 2, 2 p.p.m. Fe″; 3, 5 p.p.m. Fe″; 4, 2 p.p.m. Cu″; 5, 5 p.p.m. Cu″.

considerable amounts of precipitate are deposited, this being accompanied by a corresponding loss in tinctorial power, as shown by the following table:

Table V

Nature of sample	Dilution in $N/10$ soda	Colour		
		Y.	R.	N.T.
(*a*) 1. Double strength annatto (fresh)	1 : 10,000	6·3	0·6	0·1
2. " " (1 week in light)	1 : 10,000	5·6	0·5	0·1
3. " " (6 months in dark)	1 : 10,000	5·1	0·7	0·1
(*b*) Old annatto (2 years in diffuse light)	1 : 10,000	0·9	0·2	—
(*c*) " (1 year in diffuse light)	1 : 2,500	6·0	0·7	—
(*d*) " (1 year in diffuse light)	1 : 2,500	3·0	0·4	—

The supernatant liquid from sample (*b*) still superficially resembled a fresh extract in appearance, although the precipitate amounted to 1·5 per cent by weight of the solution.

In order to determine whether cheeses made from old solutions of annatto tend to develop colour defects (e.g. mottles, "waviness", or other discolorations), coloured cheeses were made from milk containing a mixture of annattos (*a*) 2 and (*b*), sufficient mixed colour (as found by the tintometric comparisons) being added to the vat to give the same depth of colour as the normal sample (*a*) 1, used at its usual strength in the control vat.

The cheeses were stored under various conditions (some of which were favourable to the development of colour defects) for about 6 months, but at no stage could any difference be detected between the two series of cheeses. In practice no standardization of the colour would have been made, with the result that the cheeses manufactured with the old annatto extract would have been unduly light in colour.

When the resistance towards oxidation of the old annatto solutions (*c*) and (*d*) was compared with a fresh solution by means of the accelerated oxidation test using hydrogen peroxide, or when the residue from an old sample was dissolved in $N/10$ NaOH solution to the same depth of colour as a similar solution of fresh annatto, and both solutions were allowed to bleach in diffuse light, no differences were noted in any case between the old and new samples.

It was suspected that the precipitate found in old samples of annatto (in an analogous manner to the products of fat oxidation) might possess marked pro-oxidative properties. No such properties were found, which suggested that the precipitate is a polymerized product of norbixin.

(iv) *The possible use of antioxygens for preserving annatto solutions.* The effect of sodium maleate on commercial extracts was tested but, after keeping the samples for some weeks in the light, it was found that this substance had not inhibited the loss of colour.

(v) *The differential oxidation of annatto pigments.* The "bleached" and "pink" types of discoloration (prevalent in the 1930–1 outbreak of discoloration in New Zealand cheese) were found so frequently in the same cheese, and the

merging of the two forms (giving a "dirty" colour which could not be assigned to either type) was so often observed that it seemed probable that the "bleached" and "pink" forms of discoloration were merely variants of the same defect. It has been shown by comparison of the colour extracted from the defective and normal portions of a "very bleached" cheese (on an equal-moisture basis) that only about one-half of the pigment was lost.

The defective areas of cheeses showing "pink" discoloration were also examined originally with the idea of extracting the pink pigment. On extraction with alcohol mixture, however, the discoloured portions yielded a yellow solution, and left only a faint yellow residue. The extract was compared with that obtained from normally coloured areas, and a loss in pigment was noted. The results are identical with those obtained with "bleached" discoloured cheeses, and indicate a difference in degree rather than a qualitative chemical difference between the "pink" and the "bleached" defects.

The most pronounced example of "pink" discoloration ever encountered on tintometric comparison by reflected light gave the following characteristics:

	Y.	R.	% red/total colour
Discoloured portion	4·5	4·5	50
Normal portion	9·0	4·5	33

These results indicate that "pink" discoloration is produced merely by the removal of some of the yellow component of the pigment.[1]

It has been shown in Table I that the proportion of the red in annatto precipitated on a solid medium (as in cheese) increases with the acidity, and the experiments described in connexion with Table I were extended. In one series, the annattoed filter papers were treated with a buffer solution containing 1·5 per cent, in another 6·0 per cent (by weight) of hydrogen peroxide, covered with liquid paraffin and left in the dark.

After leaving for 5 days, the relative colour intensities were found to be as shown in Table VI.

Table VI. *Alteration in colour properties of annatto pigments by oxidation*

*p*H of medium ...	4·5	4·8	5·1	5·4	5·7	6·0
	Total colour (Y. + R.)					
Initial (%)	100	100	100	100	100	100
After 5 days (%):						
(*a*) Control	74	74	77	78	79	98
(*b*) In series containing 1·5% H_2O_2	—	53	58	59	59	44
(*c*) In series containing 6·0% H_2O_2	46	35	52	44	41	23
	Red units					
Ratio red units/total (Y. + R.) units						
Initial (control) (%)	71	68	65	58	57	31
After 5 days (%):						
(*a*) Control	60	54	49	49	45	26
(*b*) In series containing 1·5% H_2O_2	—	62	60	65	61	52
(*c*) In series containing 6·0% H_2O_2	57	59	53	62	55	77

[1] The "pink" discoloration was so pronounced in the more advanced cases that before the tintometer was available, the impression was gained that extra pigmentation had actually been produced in the affected areas.

The colours of the control series were rather higher (see Table II) than is usual for New Zealand cheese, and the initial ratio red/total colour was also high, giving the filter papers a deep orange colour. In the series containing 1·5 per cent H_2O_2, there was a definite decrease in the total colour but a smaller decrease in the percentage of red, with the result that the specimens kept at *p*H 4·5–5·7 were distinctly pink, resembling in appearance this defect in Cheddar cheese. When kept for several days longer, the pink colour faded, the samples becoming bleached. The pink coloration was also shown in the series containing 6·0 per cent H_2O_2, but to a less marked extent; as seen in Table VI, this series was considerably more bleached than the other.

These results, however, have no apparent bearing on the development of "pink" discoloration in cheese. It has been noted that the "bleached" discoloured areas of some cheeses change to a "pink" form on keeping(3), but it cannot be proved that the "bleached" discoloration is an intermediate stage in the development of the "pink" defect. As shown in Table III, cheeses darken considerably when kept for 6 months after manufacture, developing considerably more red than yellow. This change is possibly responsible for the development of a "pink" tint in areas which originally appeared "bleached".

However, any other conditions favouring the presence of an increased amount of red in the annatto pigment—such as physical conditions, initially high concentration of the annatto, etc.—would favour the development of "pink" rather than "bleached" discoloration.

(vi) *Sulphydryl as an oxidation catalyst in the discoloration of cheese.* The presence of sulphydryl can be demonstrated in all mature Cheddar cheeses of normal make by means of the nitroprusside test. A typical feature of the outbreak of discoloration occurring in New Zealand cheeses during the 1930–1 season was the presence of an excessive amount of sulphydryl compounds giving a strong "sulphur" (H_2S) odour in the defective cheeses. Not only were the cheeses of the "stinker" type, but the odoriferous substance was recognized by all observers (e.g. Moir and Morgan (*loc. cit.*) and Riddet(10)) as resembling hydrogen sulphide.

The work of Hopkins and his collaborators(11) has indicated the catalytic effect of sulphydryl on certain biochemical oxidations. A preliminary test showed that the addition of a trace of hydrogen sulphide to a dilute annatto solution had but little effect on the colour until the solution was aerated, and this observation (confirming the pro-oxidative catalytic effect of sulphydryl on the atmospheric oxidation of annatto) has led to a considerable amount of study on this problem. The investigation has been carried out in collaboration with other workers at this Institute, and the observations and results will be recorded in a later paper.

Appendix

Notes on the standardization of annatto solutions for use as cheese colour

Commercial samples of annatto in use as cheese colours, obtained from local agents, have been found to vary considerably in colour intensities, as is shown by the following table:

Table VII. *Colouring properties of commercial annattos* (1 : 10,000 *in* $N/10$ NaOH *solution*)

No.	Type	Colour		
		Y.	R.	N.T.
1	—	2·0	0·7	0·1
2*a*	Double strength	6·1	1·0	0·1
b	,,	4·7	0·5	0·1
c	,,	5·1	0·7	0·1
3	—	4·1	0·8	0·1
4	—	1·3	0·5	0·1*
5	Double strength	2·9	0·5	0·1

* Stated by the agents to be used mainly for colouring "smoked fish".

From the comparison of the colour intensities in $N/10$ caustic soda solution, it will be seen that the relative intensities of the predominating yellow units vary (excluding No. 4) by over 300 per cent.

Attempts to standardize the colour at *p*H 5·5 (as in average Cheddar cheese) have been unsuccessful, as the tintometer readings obtained from such dilute acid solutions of annatto by transmitted light bear no relation to the colours in alkaline solution. In this acid medium the annatto is believed to be in colloidal suspension. It is therefore recommended that the standardization be made in alkaline solution, and $N/10$ soda has been used, and is recommended because of its ready availability.

The colour of dilute annatto solutions is not easy to estimate with the tintometer, particularly the red fraction, and the solutions are usually "over-bright", requiring the use of neutral tint. However, it is considered that workers would agree to within about ± 10 per cent on the standardization of the yellow units, and this error would not be serious.

If the colour of commercial annatto extracts were standardized, it would also facilitate the adjustment of the colour in cheeses to suit the demands of different markets. It is considered that a convenient dilution of annatto for standardized use in factories would be $1\frac{1}{2}$ oz. of colour per 1000 lb. of milk (i.e. 1 : 10,700 for normally coloured cheeses) and using annatto extracts of approximately the following colour in tintometer units (1 : 10,000 in $N/10$ NaOH solution) 4·0 Y., 0·4 R. (0·1 N.T.).

A small robust tintometer ("Limitester") adapted for testing dilute annatto solutions in the factory, equipped with a suitable range of yellow and red glasses would not be an expensive apparatus.

The keeping quality of annatto extracts. Results obtained with dilute solutions of commercial annatto extracts buffered (with acetic/acetate mixture) to *p*H 5·5 in the presence of 6 per cent by weight of hydrogen peroxide and examined after 18 hours at 65° F., merely indicated that solutions with initially high colour bleached more slowly than the others.

Owing to the destructive effects of traces of metals and light, annatto extract should be stored in light-proof glass containers, and should be kept in a cool place.

All commercial extracts have been found to deteriorate with age, and in the event of colour standardization being adopted, it would be helpful if the manufacturers marked the date of their colour determination on each container; the cheese-maker's attention would then be drawn to an old sample of annatto and, if necessary, he could readily adjust the amount of the colour to produce the required shade in the cheese.

SUMMARY

The properties of commercial annatto solutions have been examined from the point of view of the cheese-making process.

(1) The effect of initial colour strength, *p*H of the medium and distribution during manufacture are recorded.

(2) Methods are described for the extraction of the annatto colour from milk products.

(3) The effects of old annatto solutions, metallic impurities (copper and iron) and sulphydryl are shown.

(4) It is suggested that the mechanism underlying the development of "bleached" and "pink" discoloration in cheese is an oxidation process, and the effect of oxidation on annatto solutions has been studied in some detail.

(5) Suggested standards for annatto extracts for use as cheese colours are appended.

The author is indebted to Mr N. J. Ellison (who collaborated in some of the observations recorded in this paper) for useful advice and to Dr F. H. McDowall for helpful criticism.

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152. STUDIES ON THE CHEMISTRY OF CHEDDAR CHEESE MAKING

V. FACTORS INFLUENCING THE ACIDITY AND MINERAL CONTENT OF CHEESE

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The importance of acidity in determining the quality of cheese has been emphasized in a paper about to be published (1), and it has been shown that there is a general average relationship between the grader's classification of cheese as sweet or acid and the *p*H or titratable acidity of the cheese. The present paper is a study of some of the factors likely to have an effect in the acidity of cheese.

From work described in an earlier paper of this series (2) it is evident that there is a close interrelationship between acidity and mineral content in cheese. It has been shown by a number of workers (Moir (3), Wode (4), McDowall & Dolby (2)) that a high acidity in the early stages of the cheese-making process causes an increased loss of minerals in the whey and a corresponding decrease in the mineral content of the cheese. As the minerals in the cheese contribute largely to its buffering power they must play an important part in fixing the *p*H of the cheese. A study of acidity in cheese must therefore take into account the effect of variations in mineral content. The concentration of minerals also has an influence on other aspects of cheese quality, e.g. Wode (4) has shown that the calcium content of cheese is of importance in determining the body of the cheese.

The lactose content of the curd at the end of the making process is a further factor affecting the acidity of the mature cheese. It has been shown in a previous paper (5) that under normal conditions the whole of the lactose in a cheese is fermented within a few days. The lactose content of the curd when the cheese is put into the press will thus control to a great extent the change in *p*H of the cheese during the early part of the ripening process. This theory has been confirmed by Koestler (6) who has shown that in Swiss cheese the lactose content of the young cheese has a pronounced effect on the *p*H of the cheese and on the subsequent course of ripening.

Experimental methods

Manufacture of cheese

Cheese were made in the experimental factory of the Dairy Research Institute in small vats using, in most cases, 80 gal. lots of milk. The milk was treated in a flash pasteurizer, usually at a temperature of 155° F. The quantity

of starter added was 1–1¼ per cent and the proportion of rennet was 4 oz. per 1000 lb. milk. Normal cheese-making procedure was followed. As a rule one export (80 lb.) cheese was made from each vat. In cases where a number of fairly large samples were required during ripening, a number of loaf cheeses (8–12 lb.) were made. The cheese was stored at 60° F. for 14 days and at 45° F. for the remainder of the ripening period.

Analytical methods

The methods used have been described in earlier papers of this series(1,2,5,7), with the exception of the method for non-protein phosphate, while the method for estimation of lactose in curd has been modified.

Non-protein phosphate. 5 ml. of milk or 5–10 ml. of whey were added drop by drop to 10 ml. of 20 per cent trichloracetic acid in a 100 ml. graduated flask. After standing for an hour the contents of the flask were made up to the mark and filtered. Phosphate was determined in 90 ml. of the filtrate by precipitation as phosphomolybdate and weighing after heating at 160° C. The values for phosphate reported in this paper all refer to non-protein phosphate.

Lactose in curd. 100 g. of minced curd were weighed into a 600 ml. flask and 200 ml. of 0·4 NaOH added. The flask was shaken for 15 min. on a mechanical shaker and was then heated to 40–50° C. in warm water. After a further 15 min. shaking, the contents were divided equally by weight between two 250 ml. graduated flasks, a second heating being given if necessary. The second shaking of the warm mixture was found to give a much better emulsion than was obtained by the original method, in which prolonged shaking was given in the cold followed by warming before the weighing out of aliquots. Subsequent treatment of the emulsion was according to the method already described(5).

Method of expressing results

In most cases results have been expressed as g. per 100 ml. milk or whey. In cases, however, where a comparison was to be made between milk and wheys or between wheys of widely differing composition the concentrations have been expressed as g. per 100 g. water in the milk or whey. In this way the effects of differences in content of fat or other solids are automatically compensated for.

Acidity at running

It is generally recognized that the running of the whey is one of the most important stages of the cheese-making process and the running acidity may be expected to have a very considerable effect on both the acidity and general quality of the cheese. It has already been indicated that the acidity developed in the cheese milk before the addition of rennet controls to some extent the mineral losses in the whey. The following experiments were designed to show the effect of running acidity on the separation of minerals and on the quality of the cheese. In the preliminary experiments the method of manufacture used was as follows:

240 gal. of milk were treated in a large vat according to the normal cheese-making procedure until the whey acidity approached 0·155. One-third of the whey and curd was then transferred in buckets to another vat (A), the curd in the main vat being kept well agitated meanwhile. When the whey acidity in vat A reached 0·155 the whey was run. When the acidity in the main vat approached 0·17 a further quantity of curd and whey was transferred to vat B. When the whey acidity in vat B reached 0·17 the whey was run. The whey remaining in the large vat (C) was run off when the acidity reached 0·185. Normal cheese-making procedure was followed in the three vats after running. The curd in each of the three vats was salted and hooped at the same times. The whey coming off during each period was collected and sampled. Samples of cheese for analysis were taken daily for the first 3 days and thereafter at longer intervals. Results for the analysis of wheys are given in Table I and those for curd and cheese in Table II.

An examination of the results obtained from analyses of the wheys shows that the differences in acidity, lactic acid concentration and *p*H observed in the wheys at running are found also between corresponding wheys up to the milling stage. After salting the differences are small. The calcium and phosphate contents of the wheys from running to salting are higher in the vats run at high acidities. After salting the differences largely disappear. The higher calcium and phosphate losses from vats B and C are reflected in the definite lowering of the calcium content of the cheese (Table II).

The whey lost at running comprises some 90–95 per cent of the total whey lost during the cheese-making process and, as shown in Paper I of this series (2), it contains under normal conditions some 85 per cent of the calcium and 90 per cent of the phosphorus lost. Although the later wheys have a much higher mineral content their quantity is so small in comparison that variations in their mineral content can have no very appreciable effect on the mineral content of the curd. It thus becomes clear that the acidity developed in the whey before running is the chief factor in determining the mineral content of the curd and cheese.

The analytical results given in Table II show that there were no significant differences in moisture and lactose content between the cheese from the three vats. The measurements of *p*H and titratable acidity indicate that the cheese made at a high running acidity was the most acid and that made at a low running acidity the least acid. Grading results on the cheeses at 14 days and at maturity supported these conclusions. The cheese made at a high running acidity was definitely over-acid while that made at a low running acidity was sweet. Two repetitions of the experiment gave similar results as regards analyses of the wheys and the calcium content of the cheeses. The initial lactose content of these cheeses was slightly lower and the lactose was more completely fermented in the first few days of ripening than was the case with the cheeses first described. These differences were probably due to variations in the activity of the starter organisms in the cheese, a point which will be

Table I. *Analyses of wheys from vats run at different acidities*

Description of sample	pH			Titratable acidity g. lactic acid/100 ml.			Lactic acid g./100 ml.			Ca g./100 ml.			P_2O_5 g./100 ml.		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Mixed sample	6·54	—	—	—	—	—	—	—	—	0·126	—	—	0·181	—	—
Milk starter just before renneting	6·49	—	—	—	—	—	—	—	—	0·127	—	—	0·184	—	—
Whey 15 min. after cutting	6·42	—	—	0·120	—	—	0·029	—	—	0·040	—	—	0·104	—	—
Whey 90 min. after cutting	6·20	—	—	0·136	—	—	0·053	—	—	0·042	—	—	0·107	—	—
Whey at running	6·13	6·01	5·85	0·147	0·166	0·184	0·068	0·104	0·134	0·043	0·048	0·055	0·110	0·114	0·119
Whey running to drying	6·02	5·80	5·66	0·167	0·205	0·238	0·127	0·179	0·216	0·052	0·062	0·073	0·117	0·127	0·137
Whey 0–20 min. after drying	5·90	5·66	5·47	0·195	0·241	0·304	0·197	0·248	0·338	0·061	0·082	0·099	0·125	0·137	0·154
Whey 20–60 min. after drying	5·70	5·52	5·27	0·270	0·353	0·495	0·411	0·519	0·750	0·095	0·126	0·186	0·146	0·168	0·216
Whey 60 min. after drying to milling	5·40	5·23	5·11	0·498	0·645	0·828	0·920	1·07	1·41	0·198	0·266	0·345	0·211	0·268	0·333
Whey milling to salting	—	5·08	—	—	1·011	—	—	1·80	—	—	0·386	—	—	0·378	—
Whey salting to hooping	5·16	5·09	5·06	0·820	0·828	0·895	1·71	1·68	1·69	0·281	0·282	0·299	0·388	0·380	0·411
Press whey	5·16	5·13	5·06	0·962	1·045	1·036	2·01	1·96	1·90	0·342	0·319	0·324	0·418	0·452	0·443

Table II. *Analyses of cheese made at different running acidities*

Age of cheese days	Moisture			pH			Lactose		
	A	B	C	A	B	C	A	B	C
1	35·3	35·8	35·6	4·92	4·87	4·80	0·24	0·26	0·28
3	—	—	—	4·92	4·85	4·82	0·17	0·17	0·25
6	35·8	35·6	36·0	4·87	4·80	4·78	0·09	0·12	0·17
10	36·0	34·8	35·7	4·92	4·82	4·78	0·09	0·13	0·17
42	34·3	35·9	35·2	4·89	4·78	4·78	—	—	—

	A	B	C
Titratable acidity in 14 days	121	126	138
Calcium in water-free solids (%)	1·19	1·11	1·03

discussed more fully in the next section of this paper. No significant differences in lactose content between the cheeses made on the one day were observed. The grading and *p*H results again indicated the same differences in acidity between the cheeses as had been already recorded.

The effect of running acidity on the quality of the cheese was investigated more fully in a further experiment in which a larger number of cheeses were made. The general methods of manufacture were the same as those described above but only two vats of cheese instead of three were made on each day. Of each pair of vats one was a control run at normal acidity while the other was run at an acidity above or below the normal. High and low running acidities were used on alternate days. Analyses were made of the milk, of the wheys at running and of the cheese; and the cheese were graded at 14 days and at 4 months.

Analytical results are shown in Table III.

The values for the experiments described above have also been included. In all 43 vats of cheese were made. It will be observed that in every case an increase in the acidity at "running" resulted in an increased calcium loss in the whey and a lower calcium content in the cheese. In almost every case a higher acidity at "running" resulted in a lower *p*H in the cheese at 1 day old, indicating that the acidity at "running" had a definite effect on the acidity of the cheese as well as on its mineral content. The average grading scores for each lot of cheese have been set out in Table IV.

Flavour.

Fourteen days' grading. The averages slightly favour the cheese made at normal "running" acidities, but the differences are small. The cheese made at high "running" acidity were frequently commented on as having a sharp acid flavour.

Four months' grading. Here the cheese made at normal and high "running" acidities scored on the average equal points for flavour. The clean or mature acid flavour found with the cheese made at high "running" acidities was not usually objected to except where the cheese were definitely too acid. The cheese made at low "running" acidities did not find favour to the same extent. In many cases they had developed unclean flavours. Bitterness was occasionally noted and occurred most frequently with cheese made at low running acidities.

Body.

Fourteen days' grading. Cheese made at high "running" acidities were definitely inferior in body to those made at normal or low "running" acidity. The comments on cheese made at high "running" acidities frequently included references to mealy or pasty body.

Four months' grading. The average grading points indicated that the differences in body between the three types of cheese were not so pronounced

Table III. A. *Comparison of normal and high running acidities*

	Milk		Whey at running: Titratable acidity		Lactic acid		Ca		P_2O_5		Cheese: Ca in W.F.S.*		*p*H at 1 day	
Date	Ca	P_2O_5	High	Normal	High	Normal	High	Normal	High	Normal	High	Normal	High	Normal
1. iv. 35	0·130	0·159	0·185	0·17	0·169	0·162	0·069	0·063	0·106	0·104	0·85	0·91	4·82	4·83
24. ix. 35	0·130	0·175	0·175	0·16	0·110	0·080	0·055	0·049	0·111	0·106	1·08	1·11	—	—
2. x. 35	0·125	0·166	0·19	0·17	0·109	0·094	0·055	0·053	0·110	0·107	1·04	1·16	4·78	4·89
4. x. 35	0·127	0·172	0·20	0·17	0·136	0·099	0·056	0·050	0·116	0·107	1·03	1·16	4·92	4·99
8. x. 35	0·127	0·180	0·19	0·17	0·129	0·096	0·056	0·052	0·113	0·111	1·09	1·11	4·92	4·92
10. x. 35	0·126	0·178	0·19	0·175	0·120	0·101	0·056	0·052	0·117	0·111	1·03	1·06	4·78	4·85
15. x. 35	0·126	0·181	0·185	0·17	0·134	0·104	0·055	0·048	0·119	0·114	1·03	1·11	4·80	4·87
17. x. 35	0·125	0·189	0·185	0·17	0·137	0·117	0·052	0·048	0·123	0·120	1·08	1·10	4·99	5·06
21. x. 35	0·125	0·178	0·185	0·17	0·119	0·105	0·052	0·051	0·115	0·111	1·11	1·18	4·99	5·04
23. x. 35	0·126	0·179	0·185	0·17	0·122	0·095	0·054	0·050	0·113	0·111	1·09	1·17	4·92	4·92
25. x. 35	0·126	0·177	0·19	0·175	0·144	0·112	0·059	0·052	0·114	0·110	1·03	1·13	4·89	4·92
1. iv. 36	0·127	0·175	0·19	0·175	0·168	0·145	0·062	0·057	0·111	0·105	—	—	—	—
Average	0·127	0·176	0·188	0·170	0·133	0·109	0·057	0·052	0·115	0·110	1·04	1·11	4·88	4·93

B. *Comparison of normal and low running acidities*

	Milk		Whey at running: Titratable acidity		Lactic acid		Ca		P_2O_5		Cheese: Ca in W.F.S.*		*p*H at 1 day	
Date	Ca	P_2O_5	Normal	Low	Normal	Low	Normal	Low	Normal	Low	Normal	Low	Normal	Low
1. iv. 35	0·130	0·159	0·17	0·155	0·162	0·118	0·063	0·055	0·104	0·095	0·91	1·01	4·83	4·78
24. ix. 35	0·130	0·175	0·16	0·15	0·080	0·065	0·049	0·047	0·106	0·106	1·11	1·16	—	—
1. x. 35	0·124	0·174	0·175	0·155	0·095	0·077	0·052	0·050	0·109	0·105	1·13	1·20	4·96	4·99
3. x. 35	0·128	0·161	0·175	0·16	0·103	0·065	0·057	0·049	0·107	0·100	1·05	1·13	5·02	5·04
7. x. 35	0·127	0·179	0·175	0·16	0·122	0·093	0·058	0·051	0·116	0·112	1·12	1·26	4·94	4·99
11. x. 35	0·102	0·176	0·175	0·16	0·110	0·086	0·044	0·042	0·111	0·110	1·08	1·14	5·02	5·06
15. x. 35	0·126	0·181	0·17	0·155	0·104	0·068	0·048	0·043	0·114	0·110	1·11	1·19	4·87	4·92
18. x. 35	0·129	0·170	0·17	0·155	0·106	0·087	0·048	0·047	0·112	0·110	1·13	1·19	4·96	4·99
22. x. 35	0·127	0·176	0·17	0·155	—	—	0·050	0·046	0·109	0·106	1·15	1·18	4·94	4·96
24. x. 35	0·123	0·180	0·175	0·16	0·113	0·085	0·050	0·046	0·115	0·110	1·11	1·15	4·89	4·90
31. iii. 36	0·129	0·173	0·175	0·16	0·156	0·115	0·058	0·053	0·109	0·101	—	—	—	—
Average	0·125	0·173	0·172	0·157	0·115	0·086	0·052	0·048	0·110	0·106	1·09	1·16	4·94	4·96

* W.F.S. = water-free solids.

Table IV. *Average grading scores of cheese made at different running acidities*

	14 days' grading			4 months' grading		
	Flavour	Body	Total	Flavour	Body	Total
High	40·4	17·4	91·7	39·9	17·9	90·7
Normal	40·9	18·4	92·7	40·0	18·1	91·4
Normal	40·9	18·4	92·8	39·9	18·1	91·4
Low	40·6	18·3	92·5	39·4	18·0	90·5

at maturity as at 14 days. Mealy or gritty and pasty body was still frequently found, however, in the high acid cheese.

Closeness. The grading figures for closeness have not been included in the above table as they were allotted from observation of sample plugs only and therefore not reliable. An examination of photographs of the cut surfaces of the cheese yielded the following results:

Comparison of normal and low "running" acidities		Comparison of normal and high "running" acidities	
Normal definitely closer	2 cases	Normal definitely closer	0 cases
Low definitely closer	0 ,,	High definitely closer	2 ,,
Normal slightly closer	4 ,,	Normal slightly closer	3 ,,
Low slightly closer	2 ,,	High slightly closer	3 ,,
No difference	1 case	No difference	2 ,,

There appeared to be on the whole a slight tendency for a higher "running" acidity to produce a closer cheese but the differences were scarcely significant.

Total. The total scores at both gradings are, on the average, definitely in favour of the cheese made at normal "running" acidities.

A classification of the cheese, from the graders' remarks, into sweet and acid types showed that of ten cheeses made at low "running" acidity eight tended to be sweet, while of eleven made at high "running" acidity eight tended to be acid. Of eighteen cheeses made at normal "running" acidity three showed some acid characteristics and three a tendency to sweetness.

A study of the incidence of bitterness at 4 months showed that out of nine cheese made at low "running" acidity five developed bitterness while four out of seventeen made at high "running" acidity developed bitterness. These results support the conclusion reached in a previous paper(1) that excessive acidity is not the most frequent cause of bitterness.

The results given above show that the acidity at which a vat is run can have a very pronounced effect on the quality of the cheese produced. It is an important factor in determining whether the cheese will grade acid or sweet and also controls the mineral content of the cheese and has a considerable effect on the body of the cheese. It is probable that the two latter characteristics are related since, as mentioned above, it has been shown that the body of cheese is influenced by the calcium content.

Lactose in cheese

It has been mentioned earlier in this paper that the lactose content of the curd at hooping is likely to exercise a measure of control over the acidity of the mature cheese by determining the change of *p*H in the early stages of ripening. Experiments have been carried out to investigate this point and to find whether additions of lactose to the cheese milk or to the curd would have an effect on the quality of the cheese. The method of procedure was as follows:

Milk after pasteurization was run into two vats, one vat receiving 120 gal. of milk and the other half that quantity. To the smaller vat (vat 2) was added a solution of 12·2 lb. lactose in 62·5 lb. water, while to the larger vat (vat 1) was added an equivalent proportion of water (125 lb.). The lactose concentrations in the two vats were now 4·05 per cent and 5·01 per cent respectively. Normal cheese-making procedure was followed with each vat until between the milling and salting stages when one-half of the curd and whey from vat 1 was transferred to a third vat (vat 3) and treated with solid lactose which was thoroughly worked into the curd. The amount added (1·25 lb. to 77·2 lb. of curd) was calculated to be sufficient to raise the concentration of lactose in the moisture in the curd by about 2 per cent, allowing for 50 per cent loss in the whey. The curd in the three vats was salted and hooped at the normal times. Samples of whey were taken and analysed as in previous experiments and determinations of moisture and lactose were also made on the curd and cheese.

The results of analyses of wheys are given in Table V in which all concentrations have been expressed as concentrations in the water present (g. per 100 g. water). Analyses of the curd and cheese are given in Table VI.

Table V. *Analyses of milk and whey*

	*p*H		Acidity/H_2O		Lactic acid/H_2O		Lactose/H_2O		Ca/H_2O		P_2O_5/H_2O	
	1	2	1	2	1	2	1	2	1	2	1	2
Original milk	6·63	6·63	0·181	0·181	0·005	0·005	5·19	5·19	0·151	0·151	0·173	0·173
Milk + water or lactose solution	6·69	6·68	0·153	0·151	—	—	4·52	6·43	0·136	0·137	—	—
Milk + water, etc. + starter	6·59	6·57	0·165	0·166	0·017	0·011	4·42	6·50	0·134	0·135	0·154	0·154
Whey 15 min. after cutting	6·54	6·49	0·098	0·102	0·027	0·031	4·71	6·65	0·049	0·045	0·065	0·065
Whey 90 min. after cutting	6·20	6·13	0·136	0·141	0·063	0·077	4·64	6·60	0·052	0·053	0·067	0·080
Whey at running	5·89	5·75	0·167	0·186	0·127	0·156	4·52	6·45	0·060	0·067	0·089	0·096
Whey running to drying	5·56	5·46	0·255	0·277	0·273	0·261	4·32	6·39	0·092	0·099	0·116	0·121
Whey 0–15 min. after drying	5·27	5·27	0·344	0·352	0·43	0·44	4·14	6·20	0·128	0·129	0·145	0·146
Whey 15–30 min. after drying	5·32	5·27	0·462	0·467	0·71	0·71	3·76	5·77	0·191	0·192	0·184	0·188
Whey 30–60 min. after drying	5·16	5·15	0·613	0·612	1·08	0·99	3·45	5·61	0·271	0·259	0·240	0·235
Whey 60 min. after drying to milling	5·08	5·04	0·845	0·836	1·52	1·37	3·28	5·56	0·378	0·368	0·320	0·316
Whey milling to salting	—	—	—	—	—	—	—	—	—	—	—	—
Whey salting to hooping	5·06	5·06	0·884	0·916	1·73	1·64	1·28	2·74	0·298	0·309	0·409	0·427
Whey salting to hooping (vat 3)	5·01		0·887		1·69		12·82		0·332		0·402	
Press whey	5·03	5·11	1·073	1·103	2·03	1·84	1·41	3·03	0·370	0·391	0·442	0·450
Press whey (vat 3)	5·08		1·047		2·13		8·83		0·377		0·450	

Addition of lactose to milk. The distribution of the added lactose may be followed from both the whey and curd analyses. The greater portion of the lactose escaped in the whey at "running" and the concentration in the whey

Table VI. *Analyses of curd and cheese*

Description of sample	Moisture			*p*H			Lactose		
	Vat 1	Vat 2	Vat 3	Vat 1	Vat 2	Vat 3	Vat 1	Vat 2	Vat 3
Curd at running	59·9	55·2	—	—	—	—	—	—	—
Curd at drying	48·2	47·1	—	—	—	—	—	—	—
Curd 30 min. after drying	41·1	40·9	—	—	—	—	—	1·47	—
Curd 60 min. after drying	40·0	40·4	—	—	—	—	—	—	—
Curd at milling	40·2	39·5	—	—	—	—	0·53	1·09	—
Curd at salting	40·3	40·4	41·5	—	—	—	—	—	—
Curd 1 hour after hooping	34·4	34·2	35·4	—	—	—	0·42	0·83	1·08
Cheese at 1 day	34·7	33·9	34·6	4·82	4·82	4·80	0·08	0·69	0·75
Cheese at 2 days	33·4	33·6	33·8	4·83	4·77	4·70	0·07	0·42	0·61
Cheese at 3 days	34·0	34·1	34·4	4·80	4·75	4·68	0·05	0·34	0·49
Cheese at 5 days	33·2	33·0	33·3	4·82	4·66	4·63	0·05	0·21	0·42
Cheese at 7 days	33·3	33·3	33·4	4·82	4·66	4·63	0·05	0·22	0·32
Cheese at 11 days	32·9	32·4	31·8	4·78	4·56	4·58	0·04	0·16	0·35
Cheese at 14 days	33·5	32·6	32·4	4·77	4·56	4·58	0·06	0·16	0·31
Cheese at 19 days	33·0	31·8	31·8	4·80	4·61	4·65	0·00	0·14	0·23
Cheese at 44 days	31·3	30·0	30·0	4·89	4·68	4·66	0·00	0·22	0·26
Cheese at 122 days	30·5	29·4	28·1	4·87	4·66	4·58	0·00	0·12	0·13

	Vat 1	Vat 2	Vat 3
Titratable acidity at 14 days	134	154	160

at this stage was proportional to the concentration in the milk as shown by the following figures:

	Milk	Whey at running	Whey running to drying	Whey salting to hooping
$\frac{\text{Lactose/water (vat 2)}}{\text{Lactose/water (vat 1)}}$	1·42	1·43	1·48	2·14

In the later stages the concentration of lactose in the vat 2 wheys was higher than would be expected from the concentration of lactose in the milk, but the additional loss from this cause was negligible since the actual weight of lactose escaping in the later wheys was very small. The following are weights calculated from the weights of wheys and the lactose concentrations therein.

	Vat 1 (calc. on same wt. of milk as vat 2) lb.	Vat 2 lb.
Weight lost in whey at running	23·75	35·50
Weight lost in whey running to drying	1·15	1·08
Weight lost in whey after drying	0·64	1·10
Total	25·54	37·68

The actual weights fermented in each vat should not differ greatly since the acidities attained in vat 2 were but little different from those attained in vat 1.

The curd analyses show that the amount of lactose retained in the vat 2 curd was rather greater than would be expected from the concentration of lactose in the milk.

	Milling	1 hour after hooping
$\frac{\text{Lactose/water in curd (vat 2)}}{\text{Lactose/water in curd (vat 1)}}$	2·09	1·99

(Ratio for milks: 1·43.)

The addition of lactose to the milk produced only a slight effect on the behaviour of the vat. There was but little difference between the lactic acid,

calcium and phosphate concentrations in the corresponding wheys from the two vats. The only difference observed by the cheese-maker was that the rate of acid development was slightly greater in vat 2.

Addition of lactose to curd. An estimate of the amount of added lactose retained in the curd may be made by comparing the lactose lost in wheys after salting from vat 3 and vat 1. Vat 1 lost 0·087 lb. lactose while vat 3 lost 0·803 lb., the weight of curd being practically the same for each vat. The difference (0·716 lb.) may be considered the amount of the added lactose lost in the whey. Since 1·25 lb. of lactose were added to the curd, this loss amounts to 57 per cent. In a repeat experiment the loss was 51 per cent. Thus it may be concluded that 40–50 per cent of the lactose added to the curd in this manner was retained.

Behaviour of cheeses on ripening. The analyses of the curd 1 hour after hooping show the amounts of lactose finally retained in the cheese since the amount of press whey lost after that time is negligible. The cheeses from vats 2 and 3 contained much higher proportions of lactose than those from vat 1, but during the first 12 hours after hooping the amounts fermented were less than in vat 1, as shown in Table VII.

Table VII. *Rate of fermentation of lactose in cheese*

	Lactose in cheese %					Lactose fermented %				Lactose fermented as percentage of lactose 1 hour after hooping			
Vat	1 hour after hooping	1 day	3 days	19 days	122 days	1 day	3 days	19 days	122 days	1 day	3 days	19 days	122 days
1	0·42	0·08	0·05	0·00	0·00	0·34	0·37	0·42	0·42	81	88	100	100
2	0·83	0·69	0·34	0·14	0·12	0·14	0·49	0·69	0·71	17	59	83	86
3	1·08	0·75	0·49	0·23	0·13	0·33	0·59	0·85	0·95	31	55	79	88

The fermentation of lactose in the cheese from vats 2 and 3 continued to proceed slowly, so that after 3 days more lactose had been fermented than in the vat 1 cheese in which the lactose concentration had now fallen almost to zero. At 19 days twice as much lactose had been fermented in the vat 3 cheese as in the control and at 4 months a still further quantity had been fermented. The amount of lactic acid formed is indicated by the low *p*H values reached in the cheeses from vats 2 and 3. It will be noticed that even after 4 months appreciable quantities of lactose still remained in the cheese from vats 2 and 3. This incompleteness of fermentation requires some explanation. It cannot be entirely due to the retarding effect of low *p*H on the bacterial activity, since in a second experiment the same incomplete fermentation was found although the *p*H of the vats 2 and 3 cheese did not fall below 4·75 until after 4 months, while in the present experiment the control cheese reached that *p*H value after 1 day. The high concentration of lactate produced may possibly have an inhibiting influence. The most satisfactory explanation, however, is suggested by the work of Sherwood (8) who has shown that lactic streptococci do not grow readily in cheese and decrease in numbers a week or

two after the cheese is made. The lactobacilli which appear in cheese at a later date and play an important part in cheese ripening ferment lactose to only a very slight extent. Any lactose remaining in cheese after the disappearance of the starter bacteria will therefore be fermented only very slowly.

Quality of the cheese. The results of measurements of *p*H and titratable acidity indicate that the cheeses of vats 2 and 3 were definitely more acid than the control cheese. Results of gradings carried out at intervals up to the age of 4 months supported this conclusion. At 14 days and 3 months the cheese from vats 2 and 3 showed a sharp clean acid flavour and were definitely more acid than the controls. After 6 months the cheese from vat 2 still retained the same grading for flavour but the cheese from vat 3 developed a sour unclean flavour. The same behaviour was shown by cheese from a second experiment. In all cases the cheese from vats 2 and 3 showed a mealy acid body.

The results of these experiments indicate the importance of the lactose content of young cheese in determining the quality of the cheese. Cheeses with an abnormally high lactose content in the curd at hooping develop a very low *p*H on maturing and show definitely acid characteristics. While the amounts of lactose incorporated in the vat 3 cheese are considerably higher than would be possible in ordinary cheese-making practice, the amounts in the vat 2 cheese could quite conceivably be reached by natural variations in the cheese milk, while variations in the manufacturing process could alter the amounts incorporated still further. In this connexion the variations in natural lactose content and lactose/casein ratio of milk for cheese-making are subjects which invite further investigation.

Summary and conclusions

It has been shown that the acidity developed in a vat of milk at the "running" stage has a very important influence on the quality of the resultant cheese. A high acidity causes increased mineral losses in the whey and a corresponding lowering of the mineral content of the cheese, while it also has a detrimental effect on the body of the cheese. The acidity at "running" also controls to a great extent the acidity of the cheese produced. Even where the acidities at the late stages of the process are kept the same, vats of cheese made at a high "running" acidity show more acid characteristics than cheese made at low "running" acidity.

The quantity of lactose incorporated in the cheese is also of importance in determining the acidity of the cheese. An increase in the lactose content, either by raising the lactose concentration of the cheese milk or by the addition of lactose to the curd, produces a more acid type of cheese at maturity, although some unfermented lactose is still present in the cheese at 4 months after manufacture.

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153. STUDIES ON THE CHEMISTRY OF CHEDDAR CHEESE MAKING

VI. FACTORS AFFECTING THE RELATION BETWEEN LACTIC ACID AND TITRATABLE ACIDITY IN WHEYS

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(With 2 Figures)

The titratable acidity of whey from Cheddar cheese, as already shown in earlier papers of this series (1, 2), is not always a reliable indication of the amount of lactic acid which has been formed in the curd, on account of the neutralization of a portion of the acid by minerals from the curd. Measurement of the concentration of lactate ion in the whey might be expected to give a more accurate indication of the amount of lactic acid formed by the starter culture than the measurement of titratable acidity, which depends on the mineral content of the whey. A study has therefore been made of the relationship between lactate concentration and titratable acidity in wheys from milk made into cheese under varied conditions of cheese-making.

Effect of rate of development of acidity

It might be expected that with a slower development of acid there would be a more complete neutralization of lactic acid in the curd. For the same lactate concentration there would be a lower acidity in the whey from a slow vat than from a fast vat with the same milk.

The cheeses were made in parallel vats from milk drawn from a single bulk sample. Different rates of acid development were produced either by adding starters of different activities or by using different quantities of the same starter. The samples of whey collected at intervals throughout the manufacturing process were analysed for lactose, lactic acid, calcium and non-protein phosphorus, and the results were plotted against the acidity of the samples. In the first two experiments it was found that a fast vat showed a slightly lower whey acidity for the same lactate concentration than a slow vat from the same milk. In three later experiments, however, the opposite result was obtained and it became evident that any effect due to the rate of development of acidity was small and was masked by some more important effect. On account of the contradictory results obtained, the data from these experiments are not reproduced here. An explanation of the results, however, was afforded by later experiments which showed that the acidity at running

had quite an appreciable effect on the lactate-acidity relationship in wheys after running. In the experiments on slow and fast vats it had not been possible to run the vats at the same acidity without introducing complications in the later stages of the process. On investigation of the results it was found that the differences observed could be explained by the differences in "running" acidity in the vats compared. Any influence of the rate of development of acidity was evidently small in comparison with that due to the acidity at which the vat was run.

Effect of the acidity at which a vat is run

The results of the experiments on acidity at "running" described in the preceding paper of this series(3) have been utilized. Fig. 1 shows in graphical

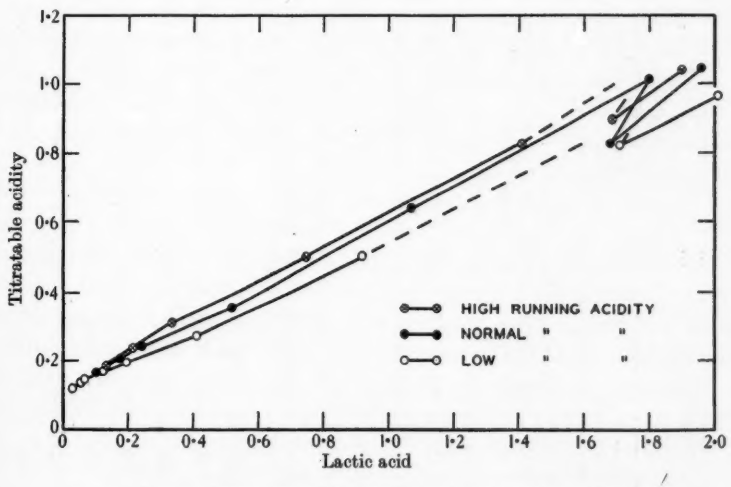

Fig. 1. Relation between titratable acidity and lactic acid in wheys from vats run at different acidities.

form the relation between lactic acid and titratable acidity in the wheys in one of these experiments. It will be observed that after the running stage there were appreciable differences in titratable acidity for the same lactate content. At all stages after running, the vat run at the highest acidity showed the highest titratable acidity for the same lactate content in the wheys. The curd in this vat contained the lowest percentage of calcium and phosphorus and it would appear that the lower mineral content resulted in a less complete neutralization of the lactic acid formed in the curd. The titratable acidity of the whey would thus be higher for the same lactate content. The repetitions of this experiment gave confirmatory results.

Table I. *Comparison of milk of high and low fat content*

Fat in milk (%) ... High test 4·20 Low test 3·45
Casein in milk (%) ... „ 2·57 „ 2·16

	Milk or whey																Curd		
	Total solids g./100 g.		*p*H		Acidity g. lactic acid/100 ml.		Acidity g. lactic acid/100 g. H_2O		Lactic acid g./100 g. H_2O		Lactose g./100 g. H_2O		Ca g./100 g. H_2O		P_2O_5 g./100 g. H_2O			Moisture g./100 g.	
Description of sample	High test	Low test	High test	Low test	High test	Low test	High test	Low test	High test	Low test	High test	Low test	High test	Low test	High test	Low test	Time sample taken	High test	Low test
Milk before addition of starter	13·38	11·79	6·63	6·68	0·148	0·140	0·168	0·156	0·009	0·008	5·48	5·16	0·143	0·119	0·166	0·160	—	—	—
Milk and starter before renneting	13·55	11·69	6·45	6·59	0·150	0·149	0·170	0·165	0·020	0·024	5·55	5·06	0·142	0·117	0·168	0·159	—	—	—
Whey 15 min. after cutting	7·08	6·66	6·51	6·47	0·099	0·107	0·105	0·113	0·031	0·035	5·50	5·07	0·050	0·044	0·078	0·090	15 min. after cutting	79·6	84·2
Whey 60 min. after cutting	7·01	6·48	6·38	6·33	0·112	0·118	0·118	0·124	0·052	0·059	5·50	5·02	0·052	0·045	0·078	0·087	60 min. after cutting	71·7	72·5
Whey 105 min. after cutting	6·93	6·42	6·21	6·13	0·134	0·146	0·141	0·153	0·084	0·098	5·39	4·95	0·056	0·051	0·082	0·091	105 min. after cutting	64·0	63·4
Whey 10 min. before running	7·07	6·47	6·06	6·02	0·150	0·154	0·158	0·162	0·117	0·107	5·43	4·93	0·062	0·055	0·087	0·097	10 min. before running	58·7	58·9
Whey at running	7·01	6·51	5·95	5·96	0·162	0·160	0·171	0·168	0·138	0·123	5·37	4·94	0·065	0·058	0·096	0·103	At running	53·9	56·3
Whey running to drying	7·22	6·79	5·68	5·63	0·222	0·237	0·235	0·249	0·264	0·269	5·07	4·72	0·089	0·085	0·114	0·126	At drying	47·1	47·2
Whey 0–15 min. after drying	7·64	7·15	5·37	5·32	0·283	0·289	0·300	0·306	0·354	0·390	5·06	4·65	0·121	0·108	0·137	0·151	—	—	—
Whey 15–30 min. after drying	7·21	6·77	5·37	5·33	0·384	0·399	0·405	0·42	0·634	0·663	4·73	4·26	0·170	0·163	0·169	0·179	30 min. after drying	41·2	41·1
Whey 30–60 min. after drying	7·51	7·17	5·27	5·27	0·50	0·51	0·52	0·54	0·93	0·94	4·47	4·10	0·233	0·222	0·208	0·221	60 min. after drying	40·2	40·4
Whey 60 min. after drying to milling	8·50	8·64	5·15	—	0·70	0·73	0·74	0·78	1·34	1·36	4·36	4·27	0·339	0·359	0·288	0·317	Milling	41·1	40·5
Whey milling to salting	25·59	—	—	—	0·86	—	1·16	—	1·91	—	3·31	—	0·529	—	0·421	—	—	—	—
Whey salting to hooping	23·97	23·06	5·27	5·13	0·77	0·68	0·90	0·78	1·87	1·80	2·22	1·50	0·317	0·252	0·400	0·380	Hooping	37·2	26·2
Press whey	22·75	19·22	5·33	5·42	0·80	0·73	0·99	0·87	2·02	1·90	2·27	1·66	0·375	0·414	0·414	0·371	—	—	—

Calcium in cheese (g./100 g. dry matter): high test, 0·90; low test, 0·89.

Comparison of milks of high and low fat content

It is a recognized fact in practical cheese-making that "high-testing" milks require the addition of a larger percentage of starter culture than "low-testing" milks and that higher acidities can be used throughout the making process without danger of producing acid cheese. An investigation was carried out to determine what differences in the milks were responsible for this difference in behaviour.

Milk was obtained from the Massey Agricultural College herd which was divided for this purpose into groups of high- and low-testing cows. The two types of milk were made into cheese in parallel vats. The percentages of starter and rennet used were the same for each pair of vats and other manufacturing details were kept as nearly the same as was consistent with the production of normal cheese. Methods used for sampling and analysis of milks and wheys were as described earlier. Determinations of moisture in the curd and of the calcium content of the cheese were also carried out. The experiment was repeated twice but as similar results were obtained only one experiment is described in detail. The results of analyses are set out in Table I.

Acid development during the making process, as was expected, was rather more brisk with the low-test vat than with the high-test vat. An examination of the data given in Table I shows that the most obvious difference between the high- and low-testing milks was that the former contained a higher concentration of cheese solids (fat, casein and minerals) than the latter. The wheys derived from the high-test milk were also more concentrated, as shown by the figures for total solids. The high-test milk was richer in both calcium and phosphate than the low-test milk but the ratio of calcium to phosphate was higher for the high-test milk. Throughout the process the wheys for the high-test vat showed a higher concentration of calcium than the corresponding wheys from the other vat. Phosphate concentrations, however, were lower in the earlier stages of the process in the high-test wheys and the calcium/phosphate ratio was higher throughout for these wheys than for the corresponding low-test wheys.

A comparison of the titratable acidities of wheys at the same lactic acid concentration gave almost identical results for the two vats except at the later stages of the process when the low-test wheys showed a slightly higher titratable acidity (see Fig. 2). This difference may possibly be due to the different calcium/phosphate ratios in the wheys. Since the high-test milk and wheys contained more calcium for the same quantity of phosphate than the corresponding low-test milk and wheys, there would tend to be a greater neutralization of the lactic acid in the high-test wheys. The differences found, as shown in Fig. 2, are in the direction required to afford an explanation of the higher acidities permissible in cheese-making with high-test milk but are, however, so small that they could hardly be significant in practice.

It would seem that the difference in behaviour between high- and low-testing milks in practical cheese-making is chiefly one of concentration. The ratio of calcium to casein was practically identical for the two milks used in the above experiment, being 0·49 for the high-test milk and 0·50 for the low-test milk. It is to be expected, therefore, that the two milks would yield curds of very similar composition except as regards fat content which would be slightly higher with the curd from the high-test milk. The calcium contents of the cheese produced from the two milks are shown in Table I to be practically identical while there was very little difference in quality between the cheese.

It seems reasonable to suppose that in dealing with a concentrated milk a larger percentage of starter culture should be added or a longer ripening

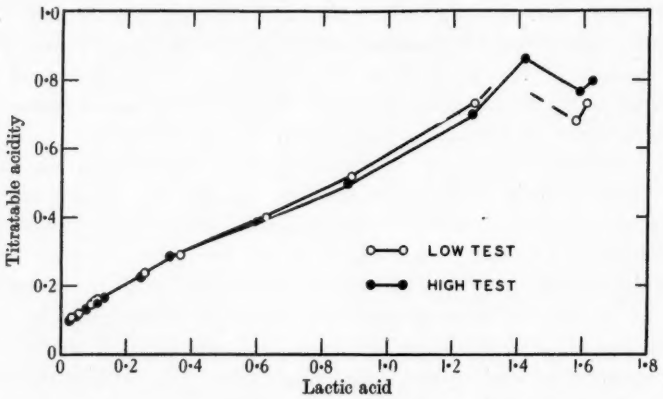

Fig. 2. Relation between titratable acidity and lactic acid in wheys from high-testing and low-testing milk.

period given so as to obtain the same number of bacteria per gram of curd and to ensure normal acid development. The more concentrated wheys obtained from such a milk would be expected to give a higher titratable acidity for the same degree of acid production in the curd.

Another factor of interest with the milks investigated was that the lactose content of the milks did not rise in proportion to the casein content as the ratio of lactose/casein was only 1·88 for the high-test milk as against 2·15 for the low-test milk. This difference is reflected in the lactose content of the wheys throughout the process and would be likely to affect the lactose content of the cheese and subsequent course of ripening in the manner indicated in a subsequent paper(3). An examination of the data given in this paper shows that the addition of lactose to the milk had no appreciable effect on the relationship between lactic acid and titratable acidity in the wheys.

Summary and conclusions

The experiments described above have shown that on the whole there is a close relationship between titratable acidity and lactic acid concentration in wheys obtained under various conditions of cheese-making. Variations in the rate of acid development or in the lactose content of the cheese milk have no significant effect on the relationship. Variations in the mineral content of the curd brought about by differences in the composition of the milk or by different acidities up to the running stage do have a certain effect. In curd with a lower mineral content there is a less complete neutralization of lactic acid and the wheys show a higher titratable acidity for the same lactate content. The differences, however, are small and affect only the wheys coming off late in the cheese-making process.

Acknowledgements

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154. STUDIES IN CHEDDAR CHEESE

V. THE EFFECT OF CHEMICAL SUBSTANCES ON THE RIPENING PROCESS

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The ripening processes in Cheddar cheese made from milk of low bacterial count are probably brought about chiefly by the enzymes of commercial rennet(1, 2) and the true lactic acid bacteria (streptococci and lactobacilli (*Streptobacteria*)(3)). In the present paper are described cheese-making experiments in which various chemical compounds likely to effect the growth and metabolism of the bacteria, or the activity of the rennet enzymes, have been incorporated in the curd at the stage of salting. The term "ripening" is considered here primarily from the point of view of protein degradation, and the amounts of nitrogen found in the various classes of protein degradation compounds are regarded as indexes of the degree of ripening.

A. Classification of the substances added to the curd

The chemical compounds added to the curd may be classified as follows:

I. *Substances stimulating, or possibly stimulating, the growth of lactic acid bacteria:* (*a*) cystine, by reason of its poising effect on the oxidation-reduction potential and possible specific catalytic effect, (*b*) potassium, sodium and ammonium citrates, which may have a specific ionic effect and also act as a carbon source for some bacteria, (*c*) sodium bisulphite, by reason of its reducing ability, (*d*) magnesium sulphate, (*e*) calcium chloride, and (*f*) small quantities of ferric salts (effect of Fe'''), these three metals behaving as activators for some enzyme systems, (*g*) dextrose, which will provide a source of energy for non-lactose-fermenting bacteria, and (*h*) autolysed yeast which has a marked stimulating action on certain bacteria, e.g. *Betacoccus* (*Leuconostoc*) and *Betabacterium*(4).

II. *Substances inhibiting or possibly inhibiting, the growth of lactic acid bacteria:* (*i*) iodoacetic acid, and (*j*) sodium fluoride, which arrest the lactic acid fermentation, and (*k*) sodium chloride in various amounts greater than normal which will depress the growth of different bacteria to different extents.

III. *Substances affecting the hydrogen-ion concentration of the cheese:* (*l*) sodium bicarbonate.

IV. *Substances possibly affecting the flavour:* namely, (*m*) diacetyl, and (*n*) sodium acetate.

B. EXPERIMENTAL TECHNIQUE

The cheese were made under good hygienic conditions from low count milk. Ordinary commercial starter (chiefly *Str. lactis* and *Str. cremoris*) and Hansen's rennet were used. The curd was divided into two or more portions at salting and the chemical substances incorporated into the curd with the salt. This method permitted the incorporation of definite amounts of the substances used and did not interfere with the normal method of manufacture. The control cheese were salted normally (1 oz. to 3 lb. curd), ripened at about 54° F., and analysed according to the methods described in a previous paper(5). Total nitrogen is expressed as a percentage of the whole cheese and the nitrogen fractions as percentages of the total nitrogen. Corrections for the nitrogen added in the substances used have not been incorporated in the tables as the quantities had no significance.

C. RESULTS

Group I. *Substances possibly stimulating the growth of lactic acid bacteria*

(*a*) *Cystine.*

It may be assumed that cystine added to the curd is largely reduced to cysteine, since although the normal cystine-cysteine potential is not accurately defined owing to the complexity of the system, the data given by Green(6) show that cysteine is unable to reduce dyes which are readily reduced in curd, viz. phenosafranine, neutral red and Janus green(7). The presence of cysteine in a medium is known to stimulate the growth of bacteria whose metabolic activities require a low potential(8). Casein contains only very little cystine(9). The effect of added cystine is shown in Table I, cheese 1–4.

Cystine in the concentrations used has thus no significant effect on the rate of ripening.

(*b*) *Potassium, sodium and ammonium citrates.*

It has been claimed by Wojtkiewicz & Inikkoff(10) that addition of citrates to the curd increased the rate of ripening. These authors however do not discuss the mechanism of this effect. The results obtained in this series of experiments are given in Table II (cheese 19, 20, 21 and 26, 32, 33, 34, 35, 54 and 57). Small amounts of citrate appear to have increased the amount of non-protein nitrogen to a slight extent, but generally speaking no significant increase in the rate of ripening was observed. Citrates thus do not appreciably accelerate the ripening of Cheddar cheese made from low count milk.

(*c*) *Sodium bisulphite.*

This reductant was added for the purpose of holding the curd at a low potential (Knaysi & Dutky(11)). Sodium bisulphite was the most efficient of the substances tried by these investigators. The effect of adding it to the cheese curd is shown in Table III, cheese 17 and 26. As is the case of cystine no stimulation was observed. Cyanide, also a reducing agent, has previously been shown to be without effect on the ripening(5). It may be concluded therefore

Table I. *Effect of cystine*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid			Total N %			Percentage of total N: Non-protein N			Total Sørensen N			Non-protein Sørensen N		
		Age in days	7	31	187	7	31	187	7	31	187	7	31	187	7	31	187
1	26·4	2 g. cystine	1·53	2·65	2·78	3·94	3·93	3·89	8·0	14·3	28·8	1·45	4·51	13·3	0·51	2·96	11·3
2	26·4	Control	1·39	2·48	2·71	3·92	3·95	3·97	7·4	14·9	28·3	1·30	4·03	13·4	0·26	2·89	11·5
3	21·5	1 g. cystine	1·54	2·14	2·92	4·20	4·21	4·14	7·50	11·5	29·3	1·83	4·35	12·4	0·33	3·00	11·2
4	21·5	Control	1·58	2·13	2·48	4·21	4·15	4·16	8·70	15·7	30·3	1·81	4·69	12·7	0·45	3·08	11·7

Table II. *Effect of citrates*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid			Total N %			Percentage of total N: Non-protein N			Total Sørensen N			Non-protein Sørensen N		
		Age in days	31	64	164	31	64	164	31	64	164	31	64	164	31	64	164
19	10	Pot. citrate 2 g.	1·73	1·96	2·09	3·967	4·089	4·050	15·5	23·0	28·8	4·5	8·9	14·0	3·7	6·4	11·6
20	10	Sod. citrate 2 g.	1·75	2·00	2·11	3·920	4·010	4·046	14·4	23·1	30·0	5·2	8·9	14·2	3·0	6·6	10·7
21	10	Amm. citrate 2 g.	1·83	1·86	1·96	3·946	4·050	4·108	15·6	22·1	29·4	4·6	8·1	13·6	2·7	6·3	11·3
26	10	Control to cheese 19–21	1·87	1·93	2·16	3·983	4·050	3·978	13·7	22·7	26·8	4·6	8·4	13·5	2·7	6·7	10·9
		Age in days	31	98	153	31	98	153	31	98	153	31	98	153	31	98	153
33	26·4	Pot. citrate 20 g.	1·54	—	1·92	3·804	—	3·883	10·9	—	29·3	3·4	—	14·7	2·5	—	11·2
34	26·4	Sod. citrate 50 g.	1·40	—	1·93	3·632	—	3·808	9·7	—	29·3	3·3	—	15·8	3·0	—	12·4
32	26·4	Amm. citrate 16 g.	1·66	—	2·13	3·880	—	3·897	12·2	—	29·7	3·7	—	15·5	2·6	—	11·8
35	26·4	Control to cheese 32–34	1·36	—	1·86	3·799	—	3·930	12·3	—	30·9	3·6	—	14·9	2·7	—	11·5
57	19·5	Pot. citrate 20 g.	—	1·81	—	—	3·844	—	—	24·2	—	—	8·5	—	—	7·9	—
54	19·5	Control to cheese 57	—	1·29	—	—	3·639	—	—	20·5	—	—	10·4	—	—	9·1	—

Table III. *Sodium bisulphite, magnesium sulphate, calcium chloride*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid			Total N %			Percentage of total N: Non-protein N			Total Sørensen N			Non-protein Sørensen N		
		Age in days	31	64	164	31	64	164	31	64	164	31	64	164	31	64	164
17	10	Sod. bisulphite 1 g.	1·91	—	2·22	3·929	—	4·053	13·9	—	27·2	5·4	—	13·0	3·5	—	10·2
22	10	Mag. sulphate 2 g.	1·94	1·95	2·12	3·970	4·040	4·138	15·3	20·7	28·6	4·7	8·2	13·4	2·3	6·5	10·5
23	10	Calc. chloride 2 g.	1·81	1·85	2·21	4·020	4·157	4·160	15·8	19·2	26·6	3·4	6·4	10·2	2·0	4·8	9·0
26	10	Control to cheese 17, 22, 23	1·87	1·93	2·16	3·983	4·050	3·978	13·7	22·7	26·8	4·6	8·4	13·5	2·7	6·7	10·9

that the poising capacity of the flora is so great that the addition of reducing substances has no detectable effect.

(*d*) *Magnesium sulphate.*

Magnesium ions have been shown to activate certain enzymes such as phosphatase (12). The addition of magnesium to the curd did not, however, increase the rate of ripening (Table III, cheese 22 and 26).

(*e*) *Calcium chloride.*

Calcium ions are known to accelerate certain enzymic processes (13). The effect of incorporating calcium chloride in the curd is shown in Table III, cheese 23 and 26.

There is apparently no increase in the rate of ripening when ionic calcium is added to the cheese. The "body" (toughness or viscosity) was increased enormously, however, in the early stages of ripening (2–6 weeks). At the end of the ripening period (6 months) the cheese had resumed a normal "body".

(*f*) *Ferric alum and ferric ammonium citrate.*

These substances may act biochemically in two ways. Thus iron is known to be a catalyst for a number of metabolic reactions. In the form of ferric ammonium citrate it has been shown to be an excellent agent for the maintenance of a medium at a high (positive) oxidation-reduction potential (11). Its effect in cheese ripening is shown in Table IV, cheese 18 and 26, 40 and 42. In the early stages of ripening no differences are detectable but in the later stages a slight inhibition can be observed.

(*g*) *Dextrose.*

The addition of dextrose may be expected to have two results: (1) an increase in the amount of lactic acid formed and hence a lowering of the *p*H, and (2) a stimulation of those bacteria able to ferment dextrose more readily than lactose (4).

The figures for cheese 36, 37 and 38 (Table V) show the results obtained when dextrose was added to the curd.

It will be seen that in the later stages of ripening there is some difference in the nitrogen distribution figures. Dextrose could not be detected after 5 months.

(*h*) *Autolysed yeast.*

It has been suggested by Allen (14) that the quicker ripening of Cheddar cheese made from market milk compared with that made from clean milk is due, at least in part, to the provision of protein degradation products for the lactic acid bacteria by the proteolytic bacteria present in market milk. Autolysed yeast has been shown to be an excellent source of nitrogen for some lactic acid bacteria (15, 4). The effect of incorporating it in the cheese curd is shown in Table VI, cheese 51, 52 and 53. The autolysate was prepared as described in reference (4) and contained about 1·3 per cent nitrogen.

No striking increase in non-protein nitrogen is observed.

Table IV. *Ferric salts*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid		Total N %		Percentage of total N: Non-protein N		Total Sørensen N		Non-protein Sørensen N	
		Age in days	31	164	31	164	31	164	31	164	31	164
18	10	Ferric amm. citrate 2 g.	1·87	1·96	3·931	4·056	13·4	23·4	4·7	12·5	3·2	11·0
26	10	Control to cheese 18	1·87	2·16	3·983	3·978	13·7	26·8	4·6	13·5	2·7	10·9
		Age in days ...	39	159	39	159	39	159	39	159	39	159
42	36	Ferric alum 5 g.	1·59	1·98	3·292	3·885	13·7	27·7	4·9	14·1	3·4	11·7
40	36	Control to cheese 42	1·75	2·04	3·365	3·936	13·1	30·5	4·6	14·5	2·8	12·2

Table V. *Dextrose*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid		Total N %		Percentage of total N: Non-protein N		Total Sørensen N		Non-protein Sørensen N	
		Age in days	35	159	35	159	35	159	35	159	35	159
37	30	Dextrose 100 g.	1·98	2·21	3·984	3·990	9·1	31·2	2·9	15·5	2·0	11·1
38	30	Dextrose 50 g.	2·18	1·97	3·952	4·001	11·5	30·1	3·0	12·2	2·2	11·1
36	30	Control to cheese 37–38	2·04	2·09	3·930	4·016	9·5	27·6	2·9	12·7	2·0	11·0

Table VI. *Autolysed yeast*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid	Total N %	Percentage of total N: Non-protein N	Total Sørensen N	Non-protein Sørensen N
		Age in days	101	101	101	101	101
52	30	Autolysed yeast extract* 400 ml.	1·97	3·929	25·1	9·8	7·0
53	30	Autolysed yeast extract* 1000 ml.	1·88	3·872	25·9	9·9	6·8
51	30	Control to cheese 52–53	1·96	3·912	23·1	9·2	7·0

* About 1·3 % nitrogen.

Group II. *Inhibiting substances*

(*i*) *Iodoacetic acid.*

This substance brings about a gradual inhibition of the lactic acid fermentation in milk (16, 17). It was incorporated at salting as the sodium salt (*p*H 6·6) after the traces of free iodine present had been removed with *N*/100 thiosulphate. The results are given in Table VII, cheese 5–10.

Although no significant differences can be observed at 21 days, the total Sørensen nitrogen is definitely lower at 7 days than in the control cheese. Titratable acidities are also lower at 7, 21 and 189 days, indicating a definite stopping of the lactic acid fermentation. In cheese 9, to which the iodoacetic acid was not added until after 29 days, normal acidities were obtained. It may be concluded therefore that the interference with the normal growth of streptococci by the addition of iodoacetic acid at salting does not affect the early production of non-protein nitrogen, but apparently delays the liberation of free amino nitrogen. After 189 days a somewhat higher non-protein figure

is evident. According to Michlin & Rubel iodoacetic acid inhibits the proteolytic enzymes of tissues (18).

Bacteriological findings in these and other cheese described in this paper will be reported later.

(*j*) *Sodium fluoride.*

This substance checks the lactic acid fermentation abruptly and thus differs in its mode of action from iodoacetic acid (19).

The effect of adding sodium fluoride is shown in Table VIII, cheese 11–15.

There is apparently no inhibition of proteolysis due to the fluoride but the titratable acidities are slightly lower than in the control cheese.

(*k*) *Sodium chloride.*

The concentration of salt is one of the most important factors controlling the characteristics of a cheese. Greater differences have been obtained in the rates of protein degradation by varying this factor than by any other method described in this paper (Table IX, cheese 16 and 26, 43–46 and 47–50).

Thus the absence of salt may increase the non-protein nitrogen by nearly 50 per cent and the use of treble the usual amount (3 oz. salt to 3 lb. curd or 6 per cent of the total weight, equivalent to 15 per cent in the aqueous phase) may decrease it by over 50 per cent. The Sørensen nitrogen is affected similarly. These differences would appear to be much greater than would be expected to follow the mere "drying out" of the curd. The moisture contents of the cheese are given (by calculation from total nitrogen) in the tables. Salt has been shown by van Dam to stimulate rennet action (20) so that the marked inhibition obtained suggests an anti-bacterial or purely physical effect rather than an anti-enzyme effect. The kinetics of the enzymic processes occurring in cheese ripening are complex on account of the number of enzymes involved and the comparatively large "substrate concentration". Thus the normally observed law that increase in substrate concentration results in increased velocity of reaction does not hold at the concentrations reached in a ripening cheese.

It is evident therefore that caution should be used in applying the results of experiments using skim milk and similar media to cheese ripening.

It has long been known that a decrease in moisture results in slower chemical ripening. It is possible to calculate roughly this "moisture" effect from the data of Van Slyke & Publow (21) (Tables X and XI).

An average moisture content greater by about 2 per cent (absolute) during the 9 months has been accompanied by an absolute increase of 14·71 per cent in the total water-soluble nitrogen.

Thus the ratio of ripening rates in the paraffined and unparaffined cheese is $\frac{27{\cdot}42}{12{\cdot}71} = 2{\cdot}16$, and the ratio of moisture contents $\frac{33{\cdot}83}{31{\cdot}65} = 1{\cdot}07$. It is evident that if the marked acceleration observed is due solely to the difference in moisture content, this factor is of considerable importance. It may be

Table VII. *Iodoacetic acid*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid			Total N %			Percentage of total N: Non-protein N			Total Sørensen N			Non-protein Sørensen N		
		Age in days	7	21	189	7	21	189	7	21	189	7	21	189	7	21	189
5	11·7	Iodoacetic acid 0·05 g.	1·39	1·73	2·05	4·074	4·074	3·905	—	10·9	32·8	1·7	2·6	13·0	—	2·1	11·5
6	11·7	Iodoacetic acid 0·15 g.	1·40	1·75	2·11	4·072	4·072	3·868	—	12·1	33·3	2·0	2·7	12·8	—	1·6	10·8
7	11·7	Iodoacetic acid 0·5 g.	1·39	1·74	2·17	4·030	4·030	3·935	—	10·3	33·3	1·9	2·6	12·1	—	1·7	10·9
8	11·7	Iodoacetic acid 1·5 g.	1·39	1·74	2·07	4·084	4·084	3·993	—	11·1	33·5	1·6	2·6	11·3	—	1·6	10·2
9	11·7	Iodoacetic acid 3·0 g.*	2·08	2·01	2·4	4·070	4·070	4·632	—	12·0	29·0	2·6	2·9	12·6	—	2·0	10·4
10	11·7	Control to cheese 5–9	1·84	2·18	2·47	4·052	4·052	4·193	—	11·9	30·1	2·4	2·7	13·6	—	2·0	11·5

* Cheese remilled and iodoacetic acid added at 29 days.

Table VIII. *Sodium fluoride*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid		Total N %		Percentage of total N: Non-protein		Total Sørensen N		Non-protein Sørensen N	
		Age in days	21	193	21	193	21	193	21	193	21	193
11	10·2	Sod. fluoride 0·4 g.	1·61	2·73	4·087	4·079	9·3	28·5	1·9	11·5	1·3	9·7
12	10·2	Sod. fluoride 1·2 g.	1·53	2·54	4·161	4·088	10·2	27·9	1·8	11·7	1·2	10·1
13	10·2	Sod. fluoride 4 g.	1·42	2·47	4·087	4·083	8·2	27·9	1·7	11·7	1·3	8·5
14	10·2	Sod. fluoride 12 g.	1·30	2·54	3·909	3·955	11·5	28·8	2·0	11·8	1·5	9·1
15	10·2	Control to cheese 11–14	1·65	2·72	4·093	4·144	10·6	27·9	1·9	11·1	1·4	8·9

Table IX. *Sodium chloride*

(Figures denote oz. per lb. curd)

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid			Total N %			Percentage of total N: Non-protein N			Total Sørensen N			Non-protein Sørensen N		
		Age in days	31	64	164	31	64	164	31	64	164	31	64	164	31	64	164
16	10	No salt	2·47	2·26	2·35	3·825	3·819	4·061	19·0	28·1	36·1	7·6	11·3	16·6	4·3	8·9	15·1
26	10	Control to cheese 16	1·87	1·93	2·16	3·983	4·050	3·978	13·7	22·7	26·8	4·6	8·4	13·5	2·7	6·7	10·9
		Age in days	—	105	—	—	105	—	—	105	—	—	105	—	—	105	—
43	31·5	1 oz. to 3 lb. curd	—	1·62	—	—	3·987	—	—	18·9	—	—	6·9	—	—	4·1	—
46	31·5	2 oz. to 3 lb. curd	—	1·67	—	—	3·832	—	—	20·1	—	—	8·4	—	—	6·3	—
45	31·5	½ oz. to 3 lb. curd	—	1·49	—	—	3·719	—	—	22·5	—	—	8·9	—	—	5·7	—
44	31·5	No salt	—	1·64	—	—	3·682	—	—	24·2	—	—	11·1	—	—	6·8	—
50	28	3 oz. to 3 lb. curd	—	1·56	—	—	4·265	—	—	9·1	—	—	5·2	—	—	3·4	—
49	28	2 oz. to 3 lb. curd	—	1·71	—	—	4·079	—	—	18·1	—	—	6·4	—	—	5·0	—
47	28	1 oz. to 3 lb. curd	—	1·75	—	—	3·746	—	—	19·7	—	—	9·0	—	—	6·7	—
48	28	No salt	—	1·82	—	—	3·752	—	—	27·0	—	—	11·8	—	—	9·7	—

Table X. *Relation between moisture content and water-soluble* N (% *of total* N)

	At 3 months		At 12 months		Increase in 9 months.	
	Water-soluble N	Moisture	Water-soluble N	Moisture	Water-soluble N	Average moisture
Normal	27·09	35·27	39·80	28·02	12·71	31·65
Paraffined	27·40	35·00	54·82	32·66	27·42	33·83

Table XI. *Effect of different concentrations of salt on ripening*

Data from reference (29)

Salt per 1000 lb. milk lb.	After 3 months		After 12 months		Increase in 9 months		Comparison of ratios of moisture content with water-soluble N ratios	
	Water-soluble N %	Moisture	Water-soluble N %	Moisture	Water-soluble N %	Moisture average	Water-soluble N	Moisture
0	34·26	38·22	51·38	34·09	17·12	36·16	1·25	1·09
1·5	32·10	35·60	45·88	31·61	13·78	33·61	1·01	1·01
2·0	31·00	35·00	44·70	31·30	13·70	35·15	Data taken as control figures	
2·5	29·92	34·43	43·52	30·99	13·60	32·71	0·99	0·99
5·0	27·70	32·62	38·19	28·61	10·49	30·62	0·77	0·93

suggested that there are at least two possible explanations: (1) that most of the moisture in cheese is "bound" so that a small apparent increase in total moisture may in reality be a large proportionate increase in water that is "free" or available for enzymic processes, (2) that the moisture content in cheese is at the critical point for bacterial growth or activity.

McDowall & Dolby (22) state that about half the moisture in curd is bound, and Hiscox (23) has observed, in expressing juice from Stilton cheese by pressure (up to 4 tons per sq. in.), that in the early stages of ripening juice could be obtained easily, in the intermediate stages with great difficulty or not at all, and in the last stages of ripening again with comparative ease.

It is evident from Tables X–XII that salt has an effect *per se* as well as by its dehydrating action on the cheese, since a *relatively* higher moisture of 7–9 per cent has resulted in a relatively higher proteolysis (116 per cent) in the salted cheese (moisture retained by paraffining) compared with only 25 per cent in the unsalted cheese. The problem of assessing the individual effects of these two factors (salt content and moisture) is difficult on account of the complex action of the salt.

Salt in cheese may influence the following: (1) the inhibition of certain types of bacteria, (2) the activation of the proteolytic enzymes of rennet, (3) the solution (early in the ripening process) of certain proteins or protein-degradation compounds, (4) the acid-base equilibria in the cheese, (5) the rate of loss of whey (moisture and soluble constituents), (6) the proportion of bound and free water. The retention of moisture by paraffining may (1) increase the viability of bacteria present, and (2) alter considerably the relative amount of *free* water present, which would permit greater quantities of substrate to go into solution.

Paraffining will also influence the gas exchange between the cheese and the atmosphere.

It is evident therefore that while the *effects* of variations in moisture and salt are well known the exact mechanisms involved are still obscure.

Table XII. *Effect of salt on rate of ripening*

(Data from present paper)

Salt % to curd	Cheese no.	Total N %	Moisture %	Non-protein N %	Ratio of average moisture*	Ratio of non-protein N
0	44	3·68	36·3	24·2	1·08	1·28
1	45	3·72	35·6	22·5	1·07	1·19
2	43	3·99	31·2	18·9	(Control)	—
4	46	3·83	33·9	20·1	1·05	1·07
0	48	3·75	35·2	27·0	1·00	1·37
2	47	3·75	35·2	19·7	(Control)	—
4	49	4·08	29·7	18·1	0·92	0·92
6	50	4·27	26·7	9·1	0·88	0·46
0	16	4·06	30·2	36·1	0·98	1·35
2	26	3·98	31·4	26·8	(Control)	—

* Calculated as in Table X, i.e. average moisture $= \frac{\text{initial moisture} + \text{final moisture}}{2}$.

Group III. *Substances affecting the hydrogen-ion concentration*

(*l*) *Sodium bicarbonate.*

Various quantities of bicarbonate were added to the curd to produce different hydrogen-ion concentrations in the cheese without affecting the making process. It is apparent that alkali may act (1) by altering the *p*H and so possibly the activity of the rennet enzymes, and (2) by allowing those bacteria normally unable to develop in cheese to grow at this increased alkalinity. The results are shown in Table XIII, cheese 24–26, 27–31, 54 and 55.

Considered as a whole the results show that the rate of ripening was not consistently affected. The *p*H values of the cheese are given in Table XV.

Group IV. *Substances possibly affecting the flavour*

(*m*) *Diacetyl.*

Diacetyl is a possible constituent of the cheese aroma and an attempt was made to find out the effect of adding this substance (17 mg.) to the cheese curd (30 lb.). The experiment resulted in a cheese that had a "stinging" effect on the tongue. The concentration of added diacetyl was about 1·7 p.p.m.

(*n*) *Sodium acetate.*

Since acetic acid is the predominating constituent of the volatile acids in Cheddar cheese, acetate was added to the curd to investigate possible effects on the flavour (cheese 40 and 41, 54 and 56, Table XIV). The experimental cheese were found to have a slight after-taste, described by some graders as metallic, but no marked effect was observed.

Table XIII. *Sodium bicarbonate*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid			Total N %			Percentage of total N: Non-protein N			Total Sørensen N			Non-protein Sørensen N		
		Age in days	31	64	164	31	64	164	31	64	164	31	64	164	31	64	164
24	10	Sod. bicarb. 10 g.	1·74	1·68	1·81	3·923	4·113	4·125	15·1	23·3	28·8	4·6	8·8	13·3	3·0	7·2	11·2
25	10	Sod. bicarb. 20 g.	1·30	1·58	1·58	3·883	3·997	3·994	13·1	20·4	28·8	5·2	8·8	13·7	3·2	7·0	11·4
26	10	Control to cheese 24–25	1·87	1·93	2·16	3·983	4·050	3·978	13·7	22·7	26·8	4·6	8·4	13·5	2·7	6·7	10·9
		Age in days	35	98	221	35	98	221	35	98	221	35	98	221	35	98	221
28	10·3	Sod. bicarb. 4 g.	1·37	—	2·30	3·840	—	4·044	12·2	—	25·8	4·0	—	14·4	—	—	10·9
29	10·3	Sod. bicarb. 8 g.	1·33	—	2·02	3·750	—	4·058	12·3	—	30·8	4·2	—	14·5	—	—	10·3
30	10·3	Sod. bicarb. 12 g.	1·22	—	1·99	3·755	—	4·135	14·0	—	29·1	4·3	—	15·9	—	—	12·0
31	10·3	Sod. bicarb. 16 g.	1·03	—	1·99	3·679	—	3·929	10·4	—	28·4	4·3	—	12·7	—	—	9·4
27	10·3	Control to cheese 28–31	1·55	—	2·56	3·798	—	4·068	14·8	—	29·8	4·2	—	14·0	—	—	9·8
55	19·5	Sod. bicarb. 50 g.	—	1·81	—	—	3·899	—	—	24·0	—	—	9·1	—	—	8·1	—
54	19·5	Control to cheese 55	—	1·29	—	—	3·639	—	—	20·5	—	—	10·4	—	—	9·1	—

Table XIV. *Alkali acetates*

Exp. cheese no.	Wt. of curd lb.	Amount of added material	Titratable acidity % lactic acid			Total N %			Percentage of total N: Non-protein N			Total Sørensen N			Non-protein Sørensen N		
		Age in days	39	98	159	39	98	159	39	98	159	39	98	159	39	98	159
56	19·5	Pot. acetate 20 g.	—	1·91	—	—	3·920	—	—	21·9	—	—	8·6	—	—	7·7	—
54	19·5	Control to cheese 56	—	1·29	—	—	3·639	—	—	20·5	—	—	10·4	—	—	9·1	—
41	36	Sod. acetate 120 g.	1·70	—	1·92	3·364	—	3·926	12·8	—	27·6	4·9	—	14·5	3·6	—	12·0
40	36	Control to cheese 41	1·75	—	2·04	3·365	—	3·936	13·1	—	30·5	4·6	—	14·5	2·8	—	12·2

Table XV. *pH values of experimental cheese*

Exp. cheese no.	Substance added	Amount added to 10 lb. curd g.	Amount added to 10 lb. curd %	Age of cheese weeks	pH	Difference from control
16	No salt	—	—	9	5·25	−0·10
19	Pot. citrate (1 H_2O)	2	0·044	9	5·17	−0·18
20	Sod. citrate (5½ H_2O)	2	0·044	9	5·31	−0·04
21	Amm. citrate	2	0·044	9	5·40	+0·05
22	Mag. sulphate (7 H_2O)	2	0·044	9	5·30	−0·05
23	Calc. chloride (6 H_2O)	4	0·088	9	5·25	−0·10
24	Sod. bicarbonate	10	0·22	9	5·47	+0·12
25	Sod. bicarbonate	25	0·55	9	5·76	+0·41
26	Control	—	—	9	5·35	—

Discussion of results

It appears that cheese is a remarkably stable system as far as the chemical ripening is concerned. Thus substances such as iodoacetic acid and sodium fluoride have no marked effect on the protein breakdown. The former has been found to inhibit the growth of lactic acid bacteria in milk at the following concentrations: *Str. cremoris* 0·001 per cent, *Str. lactis* 0·02 per cent, *Str. faecalis* 0·02 per cent, *L. casei* 0·02 per cent, and *L. plantarum* 0·02 per cent (17). In cheese this latter concentration may be considered equivalent to about 2 g. iodoacetic acid per 10 lb. curd. Thus in cheese Nos. 8 and 9 sufficient iodoacetic acid had been added to stop the lactic acid fermentation, and hence the growth of lactobacilli, which are not evident in normal cheese until a month old (3).

The small effect of these added substances may, of course, be due to the fact that the addition was not made until the curd had been formed and the rennet and acid had brought about the first stages of protein breakdown. Addition of the corresponding amounts to the cheese milk would, however, not have been possible with many of the substances on account of the interference with lactic acid formation by the starter streptococci. Therefore when considering practical applications of these results, as for example the effect of small amounts of heavy metals found in milk, the important difference between the behaviour of naturally and artificially added substances should not be overlooked.

Although there was usually no significant effect on the rate of protein breakdown, the flavour of most of the experimental cheese was adversely affected (see Graders' Report). The production of these off-flavours may be ascribed (1) to the effect of the added substance itself, or (2) to the lethal effect on the lactic acid bacteria with the removal of biological inhibition of off-flavour producing micro-organisms. In effect it has been found that the presence of heavy metals may stimulate the growth of strongly aerobic types (24).

Summary

A number of chemical substances that might be expected, or have been claimed, to affect the rate of chemical ripening have been incorporated in cheese at "salting". The effect in most cases has been insignificant. It would

appear that cheese is a remarkably stable system, the chief factors in the rate of chemical ripening being the concentration of rennet, salt and free water. The importance of the interrelationship between the last two factors has been discussed.

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GRADERS' REPORT

The cheese were judged by three disinterested expert cheese-judges

Combined results

Cheese no.	Additions	Flavour	Body	Texture
1	Cystine 2 g	8	8	8
2 C.	—	6	7	7
3	Cystine 1 g.	7	6	6
4 C.	—	7	6	6
5	Iodoacetic acid 0·05 g.	Badly off	8	7
6	Iodoacetic acid 0·15 g.	Badly off	8	7
7	Iodoacetic acid 0·5 g.	Badly off	7	7
8	Iodoacetic acid 1·5 g.	Badly off	7	7
9	Iodoacetic acid 3 g. (after 4 weeks)*	—	—	—
10 C.	—	Slightly off	6	6
11	Sod. fluoride 0·4 g.	Off	6	5
12	Sod. fluoride 1·2 g.	Off	6	3
13	Sod. fluoride 4 g.	Off	6	4
14	Sod. fluoride 12 g.	Very badly off	7	4
15 C.	—	Slightly off	6	4
16	No salt	Off	7	6
17	$NaHSO_3$ 1 g.	Off	7	7
18	Ferric amm. citrate 2 g.	Vinegary	5	5
19	Pot. citrate 2 g.	Vinegary	7	5
20	Sod. citrate 2 g.	Mild taint	5	5
21	Amm. citrate 2 g.	Mild taint	6	5
22	Mag. sulph. 7 H_2O 2 g.	Slightly aromatic	5	4
23	Calc. chlor. 6 H_2O 4 g.	Aromatic	5	5
24	Sod. bicarb. 10 g.	Aromatic	5	5
25	Sod. bicarb. 25 g.	Slightly aromatic	6	5
26 C.	—	Aromatic	7	6
27 C.	—	Strong	7	7
28	$NaHCO_3$ 4 g.	Slightly stale	8	8
29	$NaHCO_3$ 8 g.	Slight taint	8	8
30	$NaHCO_3$ 12 g.	Slightly off	8	8
31	$NaHCO_3$ 16 g.	Slightly off	6	7
32	Amm. citrate 16 g.	5	6	6
33	Pot. citrate 20 g.	6	7	7
34	Sod. citrate 50 g.	6	7	6
35 C.	—	7	7	7
36 C.	—	6	8	8
37	Dextrose 100 g.	5	5	5
38	Dextrose 50 g.	5	6	6
39	Diacetyl 17 mg.	6	6	6
40 C.	—	6	7	7
41	Sod. acetate 120 g.	7	6	6
42	Ferric alum 5 g.	7	6	6

C. = Control.

*Abnormal due to remilling after 29 days.

Graders' Report (*cont.*)

Cheese no.	Additions	Flavour	Body	Texture
43 C.	(Salt 1 oz. to 3 lb.)	8	7	7
44	No salt	4	7	7
45	Salt ½ oz. to 3 lb.	6	8	8
46	Salt 2 oz. to 3 lb.	6	8	8
47 C.	(Salt 1 oz. to 3 lb.)	6	7	7
48	No salt	3	5	5
49	Salt 2 oz. to 3 lb.	5	4	4
50	Salt 3 oz. to 3 lb.	5	3	4
51 C.	—	7	8	8
52	Autolysed yeast 400 ml.	6	7	7
53	Autolysed yeast 100 ml.	5	6	6
54 C.	—	6	7	7
55	$NaHCO_3$ 50 g.	6	7	7
56	Pot. acetate 20 g.	7	7	7
57	Pot. citrate 20 g.	7	6	6

C. = Control.

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BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. PHYSIOLOGY OF DAIRY CATTLE

I. REPRODUCTION AND LACTATION

RECENT research on the physiology of reproduction in dairy cattle has been somewhat limited. The few papers which have appeared have dealt mainly with questions of non-pathological sterility, hormones, vitamin requirements and ovarian development. Frank(1) has investigated the role of ovarian extracts in bovine sterility by administering a special preparation of the ovary in those cases in which physical examination and previous history failed to reveal the cause but in which some irregularity in oestrum was shown. Various figures are quoted to prove the efficiency of the treatment. For example, in a group of forty heifers served in spring twenty-five were found on examination in June to be non-pregnant. After treatment with the extract further examination in September showed that twenty out of these twenty-five had now conceived. In another case fifty individual animals were treated; forty-five conceived at the first service and the remaining five at the second. On the general subject of sterility two papers have appeared by Hindmarsh(2,3) and one in review form by Tutt(4,5). Hindmarsh considers that domestication and selective breeding have a definite relation to the incidence of the problem. He finds too that if the cause can be traced to malnutrition, as is sometimes the case, either oestrum fails to occur, or, if it does occur, conception does not follow even after repeated service. Adequate diet in these particular cases is a complete solution. In certain instances of sterility in cattle and in pigs Henke(6) has tried the effect of feeding sprouted oats which would supply vitamin E, but his results are inconclusive and do not appear to have any satisfactory outcome. Problems of sterility and abortion are of such importance to the dairy farmer that it may not be out of place to mention in the present review the work of Watson(7) who found the use of wheat germ oil of no value in female sterility, but claimed that, after daily administration of the oil almost throughout pregnancy, healthy living children were born to women who had previously had as many as fifteen, ten and five abortions and no normal parturition.

A section of the vitamin A requirements of cattle as related to reproduction appears in a communication published by Guilbert & Hart(8). They find that a carotene intake at the minimum level for the cow's health is insufficient to bring about the production of normal healthy calves. Of six calves born to five cows which started the gestation period with the normal reserves of vitamin A but which were given only the minimum dose during pregnancy, two developed diarrhoea and died in the first week; two developed the disease, became weak but recovered on treatment with cod-liver oil; one recovered from severe diarrhoea without treatment and one was normal. The liver of one of the calves that died contained a trace of vitamin A, the other did not. With another cow which began its gestation period with no reserve of vitamin A, the minimum dose was given daily during the first 8 months (when the animal was not lactating), and then in the ninth month the amount administered was trebled. The calf was perfectly normal. Evidently vitamin A becomes essential towards the end though not at the beginning of pregnancy.

Ovarian development has recently been studied by Casida(9) who has carried out an investigation on 273 calves ranging in age from a few days to 14 weeks. He was

able to correlate ovarian weight and degree of development of larger follicles with body weight and uterine diameter. That follicular development in the calf ovary has begun a few days after birth and is generally enhanced with age suggests that there is an increasing gonad-stimulating activity on the part of the anterior pituitary long before the time of puberty. The conclusion is drawn that the general state and physiological activity of the ovary at puberty are the result of gradual development and not of a brief process immediately before reproductive maturity is reached.

Hormones

During the past 18 months much progress has been made in the subject of hormones and their influence on lactation. Difficulties still persist in the multiplicity of names sometimes attached to the same hormone, and there can be little doubt that a valuable step in the advance of the subject would be the adoption by all authors of a standard international nomenclature. Naturally almost all the work so far published has been performed on small animals, but the results obtained in this way have added so much to the pre-existing knowledge on the subject as a whole that it becomes essential to include here a brief review of the work, particularly as hormone therapy may have considerable application to problems of lactation in the dairy cow in the near future. Experiments referred to later are already tending in that direction.

The topic which has received most attention lately is the precise nature of the action of oestrin, progesterone and ante-pituitary extracts in mammary gland development and in lactation generally. Up till recently the conclusion from much of the work was that oestrin and progesterone together cause the development of the mammary gland characteristic of pregnancy, while some hormone known variously as "prolactin", "galactin" or "mammotropin" from the anterior lobe of the pituitary is essential for the initiation and maintenance of lactation itself. Injections of anterior pituitary extracts in pregnant guinea-pigs was found by Nelson (10) to bring the pregnancy to an end and to induce lactation. When the pregnant uterus and ovaries were removed milk secretion began, but it was essential to extirpate the ovaries as well as the uterus. Removal of embryo and ovaries did not cause lactation until after expulsion of the placenta. Nelson concluded that the ovarian hormones cause growth and development of the mammary gland during pregnancy, but prevent lactation by inhibiting the secretion or the action of the necessary pituitary factor. Hypophysectomy was found by Houssay (11) to terminate pregnancy in bitches but with no occurrence of lactation, while, even in bitches already yielding milk, removal of the anterior pituitary caused complete cessation of lactation within 3–5 days. At the same time he showed that removal of the posterior pituitary did not prevent normal pregnancy, parturition and lactation even 180–254 days after the operation. Experiments of this nature were confirmed by Gomez & Turner (12) who stress the possible importance of hormones from other endocrine glands controlled by the pituitary, such as the thyroid and the adrenal cortex, as well as the entire problem of carbohydrate metabolism, which is so closely linked both with milk secretion and with pituitary function. Whether the blood sugar raising factor is identical with the lactogenic hormone has been examined by Nelson *et al.* (13) using rabbits and monkeys. They concluded (as far as the purity of the extracts would at the time allow) that the lactogenic hormone was distinct from the factor causing rise in blood sugar and also from those which stimulate growth and which appear to control thyrotropic, adenotropic and follicle activity. Lyons & Pencharz (14) have also confirmed the essential nature of the anterior pituitary in lactation by their work on intact and hypophysectomized male guinea-pigs. The intact animals secreted milk after treatment with oestrin while in the others no lactation was observed even though some growth had taken place in the mammary glands. Nelson (15) and Asdell & Seidenstein (16) have

also concluded from their work that oestrin and progesterone are definitely responsible for causing normal growth of the mamma even in the absence of the pituitary. Further interesting contributions to the subject have been made by Fremery & Spanhoff(17), Sardi(18,19), Robson(20), Gardner *et al.*(21,22), Allen *et al.*(23) and McPhail(24), using different species of animal.

From the most recent results, however, it now appears that the marked division which has hitherto been made between the part played by the ovarian hormones in causing development of the mamma on the one hand, and that by the pituitary factor in causing the actual lactation on the other, is not altogether true, for evidence published by Selye *et al.*(25,26,27) and by Reece *et al.*(28) suggests that both oestrin alone and progesterone alone, and even the two given simultaneously, are without direct effect on the mammary glands of the hypophysectomized animal. For their full activity in this direction these hormones are largely dependent on the presence of pituitary secretions. As this result is at variance with previous findings, further papers on this subject will be sought with interest. The new contention is in part confirmed by Werner(29) who studied the effect of the oestrogenic hormones and prolactin on castrated women. The former appeared to cause partial development of the mammary glands, but this was undoubtedly much enhanced by the injections of prolactin although (in this particular experiment) no lactation was induced.

Additional evidence for the inhibiting action of the female sex hormones on the actual lactation process itself has been established by Anselimo *et al.*(30) and by Snoeck(31), while Brouha & Collin(32) have discussed the relationship between lactation and hormonal equilibrium.

If hormone therapy is to advance, the assay of the active principles concerned is a matter which steadily increases in importance. In this connexion three papers may be mentioned—one by Riddle(33), another by McShan & Turner(34) and a third by Bates *et al.*(35). In the last of these the authors record the results of their assay of three hormones present in the anterior pituitaries of seven types of cattle classified according to age, sex, and state of reproduction.

The effect of oestrogenic hormones on lactation in the cow has been studied by Folley(36,37). One animal received intramuscular injections of 50 mg. oestradiol benzoate and 500 mg. oestrone over a period of 3 days, while another was given 15 mg. of the benzoate on each of five consecutive days. In both cases the total milk yield fell by about 20 per cent, the solids-not-fat in the milk rose by 10 per cent and remained at that level for a long period after the last injection, while the fat content continued to be almost unchanged. There was therefore an actual fall in the total daily secretion of solids-not-fat which was, however, considerably less than that of the fat itself, a most interesting and important observation being that a striking increase of some 400 per cent occurred in the amount of phosphatase in the milk. At its present early stage the work appears to confirm the evidence already obtained from experiments with smaller animals in which the administration of oestrogenic hormones was found to cause definite inhibition of milk secretion. But one interesting point stressed by Folley is the higher concentration of solids-not-fat which occurs and is maintained for a long time in the milk with only a few initial injections of oestrogenic hormone, even although the actual yield of milk is considerably decreased. It remains to be seen whether by combined treatment with two hormones, such as oestrone on the one hand and prolactin or thyroxine on the other, a combined and reasonably lasting effect could be produced in which both the yield of milk and the concentration of its constituents would be enhanced.

From what has already been described for other animals it will not be surprising to find that data put forward by Evans(38) proves that prolactin preparations increase the milk yield of lactating cows and goats sometimes by as much as 25–50 per cent of

the level immediately preceding the injection, an effect which disappears as soon as the injections cease and which is not altered by ovariectomy. Since the cost of hormone preparations is at present so prohibitive, Evans arrives at the important, though surely somewhat premature, conclusion that increase in milk yield would be much better obtained by genetics than by hormone therapy.

Research has also been carried out to determine the effect of thyroxine on lactation. Jack & Bechdel(39) have administered this hormone to cows at different stages in the lactation cycle and have found that its effect in causing an increase in milk secretion was greatest during the decline but some few weeks before the actual end of the lactation period. At the peak the effect was less marked while at the extreme end it was scarcely significant. Notwithstanding the increase in milk yield there was no apparent rise in the concentration of the various milk constituents, a finding which is contradicted by the work of Folley & White(40) who obtained by thyroxine administration both a marked enhancement of the total milk yield and of the majority of the main constituents with the one exception of phosphatase which, though increased in the blood serum, was very much reduced in the milk. They tend to conclude from their results as a whole that the phosphatase of milk does not come from blood serum directly but that it is synthesized in the gland itself, and that it is essential for the production of certain milk products by the gland, so that when more of the products are being made there is less of the enzyme available for excretion. Folley & White also show conclusively that the hormone does not prohibit the normal diminution in lactation as the period advances, but that this normal diminution takes place at a considerably higher level. Whether the action of thyroxine is due to its increasing the basal metabolic rate of the animal or to its stimulating a greater secretion of the essential hormone from the anterior pituitary is a question which at present remains undecided. The results of Folley & White seem to favour the former view, while Jack & Bechdel tend towards the latter. Much more experimental evidence must obviously accumulate before either conception can finally be accepted. Regarding this matter it is of interest to notice in passing that Schmidt(41) has observed an increase not only in blood sugar but also in the fatty acid content of rabbit's blood on administration of thyroxine. If this is also true for the cow it would help to explain the increased production of milk fat caused by this hormone.

Further contributions to the subject of lactation as it is affected by hormones have also been made by Jones(42), Siegert(43) and Folley(44); but for an exceedingly efficient and excellently illustrated account of hormonal action as it affects the growth and function of the udder, reference should be made to a bulletin published by Turner(45). A review has also appeared recently by Nelson(46).

Biochemical aspects

The biochemistry of milk secretion particularly with regard to milk precursors has received much attention in recent years. Nikitin(47), evidently following the work previously carried out by others, whose results have been discussed in previous reviews, compared samples of jugular blood with that from the abdominal veins of both lactating and non-lactating cows. He finds marked differences between the two in the case of the lactating but not in the dry animals, and concludes that lactose arises from blood sugar while phosphatides take part in the preparation of milk fat. In the light of recent work by others, however, this last conclusion is now generally held to be incorrect, and in fact the whole technique of using jugular blood for this purpose is no longer regarded as reliable, because, since Nikitin's publication, a paper has appeared by Blackwood & Wishart(48) confirming a suggestion previously made by Blackwood & Stirling(49) that jugular blood may be seriously affected by salivary activity and therefore cannot be taken without serious reserve for comparison with

mammary venous blood. McCay & Maynard (50) have also used this somewhat faulty technique, although they agree with other workers that phosphatides do not take part in the preparation of milk fat. A much more satisfactory method which will undoubtedly prove of great value in this direction has just been published by Graham *et al.* (51) in which the arterial sample is drawn from the internal iliac artery by puncture through the rectal wall. Once the technique has been acquired the process appears to present little difficulty and causes extremely slight disturbance to the cow. By this means 150 ml. of both types of blood may be obtained within 2 or 3 min. In some cases two samplings have been made from one cow in the same day and as many as seven in 14 days. By performing a large series of experiments to prove the value of their method, the authors have concluded that results can be relied upon provided the samplings are completed within 4 min. and that the venous blood is not taken *after* the arterial. It is essential, too, that the animal is not unreasonably disturbed, for disturbance is found to diminish the differences between the two samples. The same authors (52) have applied this process in two extensive experiments on the subject of milk precursors from which some extremely interesting results have been collected and their implications worked out, the authors rightly observing that conclusions drawn from the differences between arterial and mammary venous blood (A.-V.) must be accepted provisionally until it has been shown precisely what part is played by the lymphatic system in the changes taking place within the mammary gland. It was found that the constituents of blood which might possibly be concerned in milk production could be divided into two classes. In one of these are the substances which do not appear to be withdrawn from the blood by the gland since the (A.-V.) value for them is indefinite or insignificant. These are total cholesterol, phosphatide and ester phosphorus in either blood or plasma and also blood chloride. The other class is made up of substances for which the (A.-V.) value is very marked and significant. These are inorganic phosphorus and total fatty acids in both blood and plasma and also blood sugar. When the mean differences were expressed as percentages of the average arterial concentration it was shown in one series of experiments that the gland withdrew in some form or other 3·5 per cent of the fatty acids, 16·0 per cent of the sugar and 5·4 per cent of the inorganic phosphorus contained in arterial blood. One of the most definite conclusions from this is that the source of milk phosphorus is undoubtedly the inorganic phosphorus of the blood, and that phosphatides do not take part either in supplying this element or in preparation of milk fat by the mammary gland. From a consideration of the amount of fatty acids removed from the blood and the total amount of fat secreted by the gland, the authors arrive at the tentative conclusion that possibly 300–400 litres of blood must pass through the mammary gland for every litre of milk produced. This is equivalent to some 4 or 6 litres per min. for a cow yielding 20 litres of milk per day. When similar reasoning is applied to the glucose-lactose figures, an even greater speed is suggested, particularly when it is remembered that some of the glucose taken up by the gland is probably used for energy requirements. But regarding this estimated speed one point must be emphasized. The authors themselves state that when the cow is visibly disturbed by the sampling process, the (A.-V.) values are somewhat diminished and the experiment becomes unreliable. It is therefore conceivable that even when there are no *visible* signs of disturbance, the shock to the system caused by sampling may be sufficient to induce a reduction in the rate of milk secretion for the few minutes concerned so that the (A.-V.) values are always low, the result being an abnormally high estimate for the speed of the blood flow in order that the normal daily yield of milk may be accounted for. One of the most valuable researches which could therefore be done in the near future is the accurate measurement of blood flow in the cow by some independent method. Then if the speed estimated by Graham *et al.* were

confirmed the value of their results would be still further enhanced. With regard to the level of blood constituents and milk yield, two interesting correlations were observed in the course of this work, although more evidence must accumulate before they can be taken as being universally true. These were that the (A.-V.) values for sugar could be correlated with the arterial blood sugar content on the one hand, and with the milk yield on the other, and therefore milk yield would appear to be related to blood sugar level. If this is always true it offers a ready explanation for the effect of thyroxine in increasing milk yield and composition as found by Folley & White(40). As the authors state, further studies on animals maintained in a condition of hyperglycaemia for a reasonable time are still required for this point to be proved. Brown *et al.*(53) have recently endeavoured to contribute towards this end by studying the relationship between different sugar levels and lactose production. On causing hyperglycaemia with insulin they found that there was a marked decrease in lactose and that the general trend of the blood and milk sugar curves was the same provided the sample of milk was taken not simultaneously with, but 1 hour after, the blood sample. In one case where the blood sugar fell from 40 mg. to as low as 10 mg. the lactose production was reduced by about 20 per cent of its original figure. (It is of some interest to notice that no coma resulted at this very low blood sugar level.) Brown *et al.*(54) also tried to induce hyperglycaemia by intravenous injection of glucose and fructose, but it was found that these injections resulted in the reverse of what was required, in that hypoglycaemia was induced probably due to over-stimulation of the pancreas followed by a very large secretion of insulin, and so it was concluded that intravenous injections were of no value for the study of lactose formation. The same authors(55) then tried intra-mammary duct injections. Provided they administered sufficient sugar to exhaust the insulin supply they were able to obtain a state of high blood sugar by this means. The general conclusion was that hyperglycaemia causes a slight increase in lactose secretion, but whether results such as these obtained by the injection of such large amounts of sugar can really be compared with ordinary hyperglycaemia for this purpose is a question which for the present must remain unanswered. It is difficult to believe that an animal in that condition, in which the insulin supply has been exhausted, can really be compared with one in which a slightly high blood sugar is characteristic of the animal in its normal state. That low blood sugar causes diminished lactose secretion in cows appears therefore to be established by Brown and his colleagues, but whether high blood sugar can be correlated with increased synthesis of lactose still remains an open question, for it is conceivable that lactose production might fail to pass beyond a certain limit regardless of the height at which the blood sugar level was maintained. Tolstoi(56) was unable to find any connexion between lactose production and blood sugar level in five diabetic lactating women whether the sugar level was raised by glucose ingestion or lowered by the injection of insulin.

A further point of interest in considering lactose and one which has long remained doubtful is whether this sugar occurs in the blood of pregnant or lactating animals. With a method by which they can estimate 1–2 mg. of the disaccharide per 100 ml. blood, Hubbard & Brock(57) find that it is present in traces only, if at all, in the blood of normal and pregnant women, whereas in lactating women they estimate some 2 mg. per cent. The presence of lactose in the urine in these cases indicates that the normal renal threshold of this sugar is extremely low.

Attempted synthesis of various milk constituents *in vitro* is a process which has not been much used in the past probably due to the technical difficulties involved, and yet it is one which might well lead to much valuable information about the chemical changes which proceed within the mammary gland. Recently Grant(58) has used mammary tissue slices from the freely lactating guinea-pig in suitable physio-

logical salt solution to study the synthesis of sugar. When glucose was added to this system and the whole maintained at 37° for 6 hours appreciable amounts of lactose were formed. Such synthesis was not found, however, with galactose, fructose or mannose, and yet these three hexoses had previously been shown by Nitzescu(59) to cause increased lactose secretion when intravenously injected into the fasting animal. Grant concludes, therefore, that before they can form lactose these particular sugars have possibly to be converted to glucose by first passing through some kind of glycogen transformation. It is important to notice that there was no change from galactose to lactose, nor was there any evidence for the synthesis of free galactose, but only of galactose as an integral part of the lactose molecule. The various sugars were estimated by a differential fermentation process which has been well worked out by the author. As regards fats an abstract of the work of Michlin & Zaprudskaja(60) states that, with preparations of acetone-dried mammary tissue, synthesis of fats is increased 50–60 per cent by activators such as calcium chloride and bile, the authors suggesting that lack of knowledge as to activators and inhibitors in this reaction has been the cause of failure by others in the past to obtain fat synthesis. Work along these lines might go far towards solving some of the intricate problems of fat metabolism within the mammary gland and is a section of the subject in which development is eagerly awaited.

With regard to the enzymes of the mammary gland, Folley & Kay(61,62,63) have continued their researches on the phosphatase content. By an improved method of estimating its activity they have carried out an extensive investigation showing that this enzyme is identical with the "alkaline" phosphomonoesterase of the kidney, since the optimum *p*H, optimum substrate concentration, Michaelis constant and its variation with *p*H and synthetic powers are all identical in both cases. They(64) have also studied the changes which take place in the amount of the alkaline phosphomonoesterase in the milk during the course of the lactation period. By a mathematical analysis of their results, which include over 500 determinations on milk samples from sixty-six different cows at various times during 3 years, they conclude that the typical curve for milk phosphatase concentration as the lactation period advances is qualitatively the inverse of the corresponding curve for milk yield, the total daily elimination of phosphatase in the milk varying regularly and reaching a maximum at 180 days after parturition. From over 150 estimations of the phosphatase content in blood serum no regular changes could be detected as the lactation period advanced, a fact suggesting to the authors that milk phosphatase does not arise directly from blood but that it is synthesized by the secreting cells of the mammary gland, the total output of phosphatase bearing an inverse relationship to the activity of the gland as measured by its production of solids-not-fat. Reference has already been made to a similar conclusion arrived at by Folley & White(40) when they were investigating the effect of thyroxine on lactation.

The effect of dietary lipides on the amount and composition of milk fat has recently received attention. As far as amount is concerned McCay & Maynard(65) have re-examined the effect of feeding cod-liver oil, and have confirmed the original findings of Golding *et al.*(66) and of Golding(67) that the milk-fat content is definitely reduced by feeding the oil and that the factor responsible is not present in the unsaponifiable portion. They had previously observed the formation of muscle lesions when herbivora were fed on the oil and so they discuss whether the factor responsible for the lesions can be identical with that causing the decrease in fat secretion. Due to the paucity of evidence on either subject no conclusion on this interesting point is justifiable at present. The authors also tested the action of shark-liver oil and salmon oil but the influence of these was found to be so small and inconsistent that the effect, if any, must be very slight indeed. Also on this question a detailed series of analyses

of butterfats has been published by Hilditch & Thompson(68) who have investigated the changes brought about in the composition of milk fat by feeding three very different oils, i.e. cod liver, characterized by relatively large amounts of highly unsaturated higher acids; linseed, containing mainly linoleic and linolenic acids; and rape, of which some 50 per cent consists of erucic acid. Cod-liver oil caused the usual decrease in milk-fat secretion while the other two did not. On making a comprehensive analysis of the different butterfats produced, it was found that cod-liver oil caused a reduction of some 50 per cent in the molar content of each of the lower fatty acids (C_4–C_{10}) and of about 30 per cent in that of myristic and stearic acids. The molar concentration of oleic acid on the other hand was greatly increased, while that of the C_{20} and C_{22} highly unsaturated acids rose from 0·8 to 5·2 per cent. In view of this latter increase it was strange to find that palmitoleic acid did not appear to pass into the milk. With linseed oil the only marked effect was again a considerable increase in the proportion of oleic acid, while there was no indication that fats containing linolenic or unusual amounts of linoleic acid had been secreted in the milk. With rape oil the oleic-acid content was again enhanced and there was some 4 per cent of erucic acid in the butter fatty acids. The relationship between fully saturated glycerides and the proportion of total saturated acids was normal in all three cases. The theory is put forward by the authors that this very marked effect of cod-liver oil may be due to the selective adsorption of the highly unsaturated compounds by the enzymes which prepare the milk fat in the gland and that the normal action of these enzymes is thus very much hindered. It will be of interest to see if this theory is confirmed by future work. That this effect is not confined to the cow has been shown by Madsen *et al.*(69) who have found cod-liver oil to have a reducing action on the amount of milk fat secreted by other animals such as the guinea-pig, rabbit, goat and sheep.

Riemenschneider & Ellis(70) have made an analysis of goat milk fat by the ester distillation process. Their method differs from that of Hilditch & Thompson in that they do not first fractionate their acids into solid and liquid portions. Among the more interesting results is the finding of decenoic, tetradecenoic and palmitoleic acids. On account of their bromination experiments the authors believe that linoleic acid (now of great importance from the point of view of essential dietary constituents) is absent, but it cannot be too strongly stressed that bromination experiments in themselves are quite inadequate for the detection of this acid, the presence of which should always be established by oxidation to the corresponding hydroxy-derivative. On feeding cotton-seed meal to goats, Riemenschneider & Ellis(71) found that there was an increase in capric, lauric, myristic and stearic acids at the expense chiefly of palmitic and oleic.

Biophysical aspects

During the past 18 months several bulletins dealing with some of the more physical and anatomical aspects of lactation problems have appeared from the Missouri College of Agriculture and written mainly by C. W. Turner. The anatomy of the mammary gland of the cow and of several other animals has been fully treated by this author(72) in a very attractive and comprehensive way. His book should certainly become a standard work of reference for all whose researches are connected with the mammary gland. The smaller bulletins are also excellently written and illustrated, but they possess one disadvantage in that the author frequently gives no clue to the date and source of the information upon which he draws. Otherwise he presents a very clear exposition and concise summary of the latest theories on the subject. The general nature and function of the udder is discussed by him(73) under such headings as its internal structure, its blood supply, the lymphatic and nervous systems and the mode of milk secretion. The last heading is treated in even greater detail in a later

paper, again by Turner(74), in which are summarized the conclusions which he has drawn from evidence published during the past few years. Apart from a few minor constituents which reach the milk directly, the main substances are now believed to be synthesized in the epithelial cells lining the alveoli of the udder, each cell being capable of building up protein, fat and lactose. Then by a rupture of the cell walls or to a lesser extent by direct diffusion the milk is discharged from these cells. As the period after each milking advances, the pressure in the udder naturally rises and this has two effects. It causes reduction of the blood flow in the capillaries and makes rupture of the epithelial cells more difficult. Consequently the rate of milk secretion decreases as the period after milking lengthens, and also as the rupture of the cells becomes more difficult substances such as fat, and to a lesser extent casein, which are not in true solution in the milk, are excreted to a correspondingly reduced degree, while albumin and lactose are comparatively unaffected. Thus when periods between two milkings vary beyond a certain limit, the milk after the longer period will be relatively poorer, particularly in fat. Confirmation of this finding as it affects milk secretion as a whole is obtained from the work of Graham *et al.*(52), who have shown that the amount of phosphorus withdrawn from arterial blood by the mammary gland reaches a maximum 4 hours after milking and then steadily declines as the udder fills. Turner also deals in the same bulletin with the actual act of milking, and points out that, if the animal is not unfavourably disturbed for any reason, handling the teats should cause an increase in pressure in the udder due to the contraction of certain muscles lining the ducts, which greatly facilitates the milking process, for which the co-operation of the undisturbed animal is therefore required.

The anatomy of the mammary gland has been studied from another view-point by Chiodi(75), who has tried to discover some correlation between the actual nature of the gland and the yield of milk produced. He came to the conclusion that where high milk production was characteristic of the animals, the quantity of epithelium glandular tissue was greater compared with the connective tissue than in those cows from which lower yields were obtained. Clinical investigations with regard to udder development and the onset of milk secretion in animals in their first pregnancy have been made by Schulze(76).

The involution of the mammary gland and its bearing on the decline in milk yield during the lactation period is another anatomical and physiological process of immense importance, particularly now that by hormone therapy it might be possible to control or even prevent the involution in a way which has not hitherto been thought possible. The whole matter is therefore being examined by Turner & Reineke(77) who have so far shown that there is definitely a gradual involution in the case of the goat as the lactation period advances, but that the involution proceeds more slowly than the actual decline in milk secretion. They also find that when milking is stopped 30 days after parturition, the involution goes on progressively, being almost complete in 48 days, while after 5½ months only one duct system is left comparable to that of the virgin gland. The stimulating action of milking was found to retard involution towards the beginning of the lactation but to have little effect towards the end. The authors conclude that proper stimulation with hormones might have most beneficial results in retarding the normal decline in lactation. Such a suggestion will cause future contributions on this important scientific and economic problem to be followed with much interest. Working on the mammary gland of the rat Selye(78) has decided that involution after weaning is conditioned by the withdrawal of the nervous stimulation of sucking rather than by accumulation of the products of secretion.

Clinical chemistry of dairy cattle

During the period covered by this review further advances have been made in work on the metabolically active constituents of blood. Allcroft & Green (79) have continued their researches on calcium and magnesium contents, and have published some valuable data concerning these elements in cow's blood both in health and disease. They find that serum calcium ranges from 8·65 to 11·65 mg. per 100 ml. while the corresponding figures for magnesium are 1·85–3·17 mg. In generalized tuberculosis the magnesium content was definitely low in thirty-two out of thirty-five cases, while in lactation tetany, serum calcium ranged from 4·7 to 10·8 mg. and magnesium from 0·3 to 1·1 mg. It was also found that in five cases of milk fever serum calcium varied from 3·8 to 6·8 mg. while the magnesium appeared to remain within the normal limits. Some cases possessing both the typical hypomagnesaemia of lactation tetany and the typical hypocalcaemia of milk fever shared the usual symptoms of both diseases. Godden *et al.* (80) have added to these results by an extensive investigation on the variations in serum calcium and magnesium and the partition of the calcium in normal parturition and in milk fever. Symptoms of the disease were found to be preceded by a much steeper fall than usual in the total serum calcium before and at calving, the fall in calcium taking place chiefly in the fraction capable of being adsorbed on barium sulphate. As the milk fever disappeared, total serum calcium and magnesium increased together.

"Transit tetany" in pasture-fed horses (notably in suckling mares) has been studied by Green *et al.* (81) from the point of view of serum calcium and magnesium. They compare the condition with typical "grass tetany" so often occurring in the cow after passing from winter housing conditions to pasture feeding, and show that it is readily distinguishable from "milk fever". Sjollema (82) has examined the nature of the tetanic convulsions from which calves sometimes die when fed for some months on milk either alone or with very little supplement. The content of blood minerals is abnormal, and the disorder is said to be due to excessive protein intake. When the symptoms first appear, the author recommends that the protein consumption should be restricted, and when the calcium and magnesium contents of the serum are depressed, $CaCl_2$ and $MgCl_2$ may be injected intravenously with beneficial results.

Evidence has been collected by Landingham *et al.* (83) showing that age and level of phosphorus intake have no effect on the whole blood calcium of growing heifers.

The subject of calcium, phosphorus and magnesium ions in the body, their absorption, their fate in the blood stream, and their variations in the blood in different physiological and pathological conditions, has recently been reviewed by Schmidt & Greenberg (84).

Seasonal variations and other factors affecting milk secretion

During the past 2 years much work has appeared in connexion with climatic and seasonal variations and their effect on milk secretion. Unfortunately the problems as a whole still remain unsolved. By making more than 3000 butterfat estimations over a period of 5 years on the milk of individual animals in a herd of 40–50 cows, Oxley (85) has concluded that at all times of the year morning samples contain a higher fat percentage than the corresponding afternoon samples, the minimum seasonal value occurring during the January to March quarter for afternoon milk and during April to June for morning milk. By analysis it was shown in almost every case that the seasonal differences were significant in the case of the morning milk but less so in that of the afternoon. The author suggests that the cause for the larger differences observed between the first and second daily milkings from April to June is that the cows were out to grass during the day but housed at night for a portion of

the time. Golding *et al.*(86) have published their final series of results in their study on the milk from a typical Shorthorn herd. In each of the 5 years there was a fall in the fat content of the morning milk in the spring, while during the drought of 1933 a larger fall took place in the percentage of solids-not-fat in both morning and evening milk than has previously been recorded. Titratable acidity, formol titration, ash, calcium and phosphorus were all found to be higher during the winter months (October to February), and decreased again during the spring and summer. The decrease was closely allied with the fall in phosphorus content. In the same series of experiments Davies(87) observed that there was no seasonal variation in the content of iron and copper in milk samples taken once monthly over a 2-year period, and from his results as a whole he concluded that the fall in solids-not-fat, already referred to, was made up of two factors: (1) a rapid change in lactose content which was sudden both in its appearance and disappearance, and (2) a more gradual change in the amount of protein present. The chloride content varied inversely with that of the total solids-not-fat.

The effect of high environmental temperature on the secretion and composition of milk has been studied by Bartlett(88) who has shown that the solids-not-fat as well as the fat content itself are slightly reduced by high temperature. When the cows were kept at 80° F. the milk yield dropped by 0·16 lb. per day and the fat percentage by 0·04 (neither figure being significant), while the solids-not-fat decreased significantly by 0·153 per cent. Owing to the fact that the experimental decrease in milk quality was so very small while his experimental temperatures were continuously higher than English summer conditions, Bartlett assumed that temperature was by no means the only factor involved. Indeed the influence of other factors is stressed by Becker & Arnold(89) who have collected data for a herd of Jersey cows over a long period including almost 300 lactations. By averaging the butterfat percentage for months after calving and also for calendar months, two sets of figures were obtained. These showed that although fat percentage varied inversely with temperature—an average increase of 10° F. between monthly mean temperatures of 57° and 81° causing an average decrease of 0·31 per cent—yet internal or physiological factors such as the stage in lactation had considerably more effect than environment.

That many factors are involved is stressed by work from Africa, South America and Hungary. Hartley & Baker(90) have had the unusual opportunity of investigating these questions in a climate of great extremes. They did not find, like Oxley, that at all seasons of the year the morning milk was richer in fat than that of the evening. On the Samura Government Farm in Nigeria this was only true during the wet season when the humidity was as high as 68. During the dry period of the year when the humidity reached the low figure of 15 the reverse was found to hold, a fact which remained unexplained by the observation that the amount of evening milk was slightly less in the dry season than it was when the humidity was high. The solids-not-fat were not investigated in such detail but the percentage was consistently somewhat greater in the morning milk. Some evidence is put forward by the authors to suggest that in their particular case the matter is definitely an involved climatic effect and that it is independent of alterations in diet, in age, or on the stage of lactation. The work of Becker & Arnold, however (see above), would undoubtedly suggest that stage of lactation is at any rate of sufficient importance to be taken into account, although climate and seasonal variations of such extremes as those of Samura have, in all probability, a much greater influence in the present instance. Additional work which may be cited is that of Deger(91) in South America and Czukas(92) in Hungary who both state that morning milk is poorest in fat content. When three milkings were carried out daily (6 a.m., noon and 6 p.m.), Deger found the highest fat content in the noon sample, while Czukas observed that the morning milk contained 30 per cent less

fat than the noon milk and 40 per cent less than that of the evening. Deger suggests that the large drop in temperature from 8–9° C. during the night causes retention or consumption of fat by the animal to maintain body temperature, resulting in a low fat content for the morning milk. But obviously this explanation cannot be applied to the opposite findings recorded by certain workers in other countries and already discussed. In fact it is more likely that the solution of the problem lies in the theory propounded by Turner (74) and already cited where he explains the occurrence of low fat content after a long period without milking as being due to the inhibiting action of the pressure developed within the udder on the process of secretion. Obviously much evidence has yet to accumulate before the various factors involved in this problem can definitely be traced and their precise influence analysed. It appears that much might be gained by comparing and contrasting results collected from different countries where the climatic conditions show extremely wide variations.

In connexion with seasonal changes it is of interest to notice that Hileman & Courtney (93) have found that the amount of lipase secreted in milk and definitely not of bacterial origin, a matter of some importance where the storage of milk products is involved, reaches a minimum in early summer and a maximum in winter, and that some is present throughout the year. They confirm the view already held that one of the factors controlling lipase secretion is certainly the lactation cycle, for, apart from the season of the year, as the lactation advances there is a tendency for the amount of lipase to increase.

Another interesting effect of seasonal variation is that reported by Booth *et al.* (94) where they find that the ability of butter fatty acids to absorb in the region of 230 m. is more than doubled during the summer months when the cows are at pasture. This might be due to the presence in butterfat of small amounts of hitherto unknown acids having very high extinction coefficients, or, alternatively, that one or more of the known acids in butterfat are present in a form having a greater absorptive ability than the particular isomer of the same acids present in the foodstuff. Evidence that some such change can take place in the organism is supplied by Dann *et al.* (95) who show that a similar alteration can also be brought about in certain fatty acids by boiling with alcoholic potash.

Gooch (96) who has collected records on 99 cows having a total of 679 lactations finds that the season in which the lactation begins has a definite influence on the trend of milk production during that lactation, the more persistent milkers beginning in the winter months. She also concludes that an important value for deciding on the cow's ultimate worth as measured by the total yield of milk is the amount given by the animal at the *beginning* of its lactation. With regard to the length of the dry period and its effect on milk production, 31–60 days have been found by Arnold & Becker (97) to allow maximum yields with Jersey cows in Florida, periods outside these limits having a tendency to cause lower total yields and an earlier decline in milk production.

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II. NUTRITION

Physiology of digestion

The process of evacuation of the rumen is a subject of immediate importance in the feeding of ruminants. Columbus(1) has investigated this matter in great detail. Within 2 hours of ingestion the test meal was uniformly distributed in the rumen of sheep and goats. The major portion of the meal left the rumen in 24 hours and completely in 8–12 days. Faecal excretion of the test meal commenced in 12–15 hours, reaching a maximum at 2 days. The excretion was complete in 15–20 days. The rate of passage of the food through the alimentary tract was accelerated by green food. Fasting before the test meal delayed food movement at first but the total time of passage was unchanged. With no roughage the emptying time of the rumen was delayed to 17–20 days and complete excretion to 22–23 days. With lambs milk passed straight into the abomasum and excretion reached a maximum in 24 hours. The rate of passage of foods has been used to determine the oesophageal reflex action(2). This has been gauged by the rate of excretion of stained straw particles; the more effective the reflex the more rapid the excretion. In young ruminants dry foods had no effect on the reflex action whereas liquids caused a marked reaction. In adult animals the reflex was seldom obtained. Lenkeit & Columbus(3) also studied regurgitation from the abomasum into the rumen. Lambs (3–5 months old) were given 500 ml. of water or milk containing KI and rumen contents and urine were tested for iodine. The liquid went directly into the abomasum, yet in 1 hour iodine was found in the rumen. With sheep (> 1 year old) the liquid passed directly into the rumen. No absorption from the rumen could be found. With a copper sulphate solution Ross(4) found with adult sheep that the liquid went directly into the abomasum through the oesophageal groove. The closing of the groove is apparently a reflex elicited by contact of the solution with the mouth and pharynx. Passage of the solution directly into the oesophagus did not close the groove.

The effect of different types of milk on rate of passage has received attention. Sheehy(5) noted that many pail-fed calves had diarrhoea and subsequently died, due apparently to the formation of a dense white curd in the abomasum. With milk from newly calved cows, or with milk and water (50 per cent), a more flocculent curd was found. The milk passed more readily through the alimentary tract and no hard curd was noted in the abomasum. Fat is also important for the production of a friable curd which will readily leave the true stomach(6). With separated milk the curd was viscous and stringy and took much longer to leave the abomasum than milk with 6 per cent fat. The effect of heat treatment on the curd tension of skim milk has been studied(7). Boiling for 3 min. caused a lowering of curd tension by 80 per cent, pasteurizing for 30 min. at 142° F., a lowering of 20 per cent, while autoclaving for 15 min. at 242° F. gave no curd tension. On feeding, 2 litres of raw skim milk left the abomasum in 12–18 hours, whereas the boiled and autoclaved took only 8–12 hours. It is suggested that this is due to the lowered curd tension with a consequent increase in surface for enzyme action.

With fibrous foods, such as straw or hay, there is a primary digestion in the rumen before the food is passed into the abomasum. The rate of digestion has been studied(8) and it was found, by examining rumen contents at 2-hour intervals after feeding, that an increase in fibre occurred. This was due to the passage of protein into the succeeding stomachs. This agrees with the experiments of Kick & Gerlaugh(9). They noted that, with finely ground hay, the protein tended to leave the rumen more rapidly than the fibre. With coarse hay no such difference was noted. They also found that the number of chews, time spent on rumination, number of boluses and number of chews per

bolus were less the finer the hay. Further confirmatory evidence is shown in experiments in which hay was fed and the rumen contents analysed at regular intervals(10). An increased percentage of nitrogen and fat was noted, but this seemed to be due to a loss of carbohydrate by fermentation.

The effect of roughage on the digestibility of a normal ration has been examined by Mead & Goss(11). Heifers were reared till 18 months old on a concentrated ration with no roughage, while a control group had the same ration together with 14 per cent of sulphate wood paper pulp. No difference was found in total digestibility or in the clinical conditions of the two groups of animals.

Studying bacterial growth in the rumen, Pochon(12) isolated one organism, *Plectridium cellulolyticum*, and grew it in a neutral medium. In neutral media it destroys cellulose with the formation of fatty acids. In acid media, however, the bacterium forms a cellulase which converts cellulose into glucose. He suggests that, *in vivo*, proliferation occurs in the rumen where cellulose is split to form fatty acid. In the abomasum growth will cease, due to the acidity, and the cellulase then converts the cellulose into glucose. In a later paper(13) he claims to have found a facultative anaerobe, which is cellulolytic when grown anaerobically in an alkaline medium low in nutrients but rich in cellulose. The end products are formic, acetic and propionic acids, together with alcohol. In acid media, *p*H 4–5, glucose is formed but the cellulolytic activity greatly reduced.

The possibility of cellulose digestion being partially due to a symbiosis of bacteria and Protozoa has been suggested by Pierantoni(14). In termites the posterior intestine flagellate Protozoa are inhabited by symbiotic bacteria. Both are responsible for the digestion of cellulose. He suggests that this may also occur in ruminants.

Regarding enzyme digestion, Neseni(15) examined the tissues of foetuses of cattle and sheep for enzymes at different ages. He found that the enzymes did not appear at the same time in all the tissues examined (liver, stomach, pancreas, intestines and salivary glands). That cellobiase is not a constituent of the rumen mucosa, but is of alimentary origin, seems to follow from the experiments of Antoniani(16). He noted that neither the rumen mucosa nor aqueous extracts, made by prolonged maceration in the presence of toluol at 37° C., hydrolysed cellobiose, although cellobiase was readily found in the expressed fluid.

Analyses of the abomasal contents of two sheep(17) have shown that the *p*H and titratable acidity of the gastric juice are not closely correlated. This appears to be due to the presence of large amounts of organic acids. The only important enzyme present was pepsin.

Energy metabolism

Studying the records of forty-two cows with 136 lactations of more than 200 days, Smith & Rice(18) calculated the average efficiency of feed utilization, basing their results on the total digestible nutrients in the feed and milk. The average efficiency was approximately 30 per cent. The level of efficiency appeared to be inherited and was maintained from lactation to lactation. Although a positive correlation existed between the level of production and efficiency, no correlation existed between age, weight or amount of feed and efficiency. A similar study was undertaken by Brody & Procter(19). By analyses of food intake, milk yield and live weight changes, they found an equation showing the distribution of digestible nutrients between milk, maintenance and live weight gain, 0·305 lb. of digestible nutrients gave 1 lb. 4 per cent milk, and 2·1 lb. of digestible nutrients gave 1 lb. of live weight gain. The digestible energy cost of milk production was 1·64 times the energy of milk, while the energy cost of maintenance was 2·4 times the basal metabolism. The authors assume that maintenance cost increases *not* with simple body weight but with body weight to the power 0·73. In a later paper Brody & Ragsdale(20) give a table and chart from which,

knowing the weight of animal and the yield of 4 per cent milk, the efficiency of the cows can be found.

The effect of nutrition on energy metabolism has received much attention in the past 2 years. Although the experiments have been carried out using rats, the work is of such importance from the point of nutrition of farm animals that a brief résumé will be given. It must, however, be stressed that the results cannot be used directly with ruminants, although it is probable that the broader principles will hold. Perhaps the value lies more in the type of experiment which could profitably be carried out with ruminants.

The effect of different planes of nutrition, from fast to full feed, on heat production has been studied(21). The results, showing a progressive increase in the rate of rise of heat production with increase in food consumption within certain limits, confirm those of earlier experiments(22,23,24,25). The effect of protein, fed at four planes (10, 15, 20 and 25 per cent), on growth and metabolism was examined(26). The results show that with increased protein intake an increased retention of both protein and energy-producing substances takes place, with a decrease in the amount of fat gained in proportion to protein. Although an increase was noted in the energy of the urine as the protein increased, the metabolizable energy remained constant, due to a decrease in the energy content of the faeces. The increase of protein in equicaloric diets appears to improve the nutritive balance of the food but no apparent change in basal heat production per unit of surface area is found. Under normal conditions of freedom of activity an increase in protein produces a diminution in the total heat production of the animals. In a study of the effect of a vitamin B-deficient diet, McClure *et al.*(27) noted various depressing effects on the general metabolism of the body. The quantities of fat and energy gained decreased markedly together with a diminution in the efficiency of utilization of metabolizable energy for body gain. The oxidative processes of the body were markedly depressed while the energy output in the urine and in heat increased. The effect of a cystine deficiency was not so marked, only a slight decrease in the amount of energy stored being noted(28).

Using steers and cows, Kriss(29) noted a close correlation between the metabolizable energy of a ration and the percentage of heat lost by evaporation of water, a change in one producing a corresponding change in the other. This effect was very much more marked when the ration was fed above the maintenance level. Clipping of the animals reduced but did not entirely eliminate the effects of the plane of nutrition on the manner of heat disposal.

In an attempt to estimate the basal metabolism of dairy cows, Benedict & Ritzman(30) made determinations on the 4th and 5th day of fasting following different rations. In the same animal variations up to 90 per cent were found, with no apparent cause. They suggest that the determination of the base-line should be carried out for each experimental period. The effect of walking on energy metabolism has been studied in detail by Hall & Brody(31). The percentage increase in heat production of walking over standing varied directly with speed and was shown by $Y=74\,S$, where Y was the percentage increase in metabolism at speed S miles per hour. When the energy cost of standing at rest was not included, the net energy expense of walking, i.e. walking over standing, was per unit of live weight and unit distance walked independent of speed. When the energy cost of standing was included, the *total* energy cost of walking decreased with increasing speed. The decrease was exponential, approaching the *net* energy expense of walking as a limit as shown by $Y=44e^{-0{\cdot}268S}+33$, where Y is the calories per 100 lb. per mile, S the speed and 33 the net energy expense of walking per kilo-calorie per 1000 lb. live weight per mile. These relations were apparently independent of the live weight of any given species, unless the animals were too fat.

An interesting paper by Wiegner & Grünigen (32) shows that there exists a difference in the energy metabolism of fattening animals and those either growing or producing milk. They found that the energy lost as heat was less during protein anabolism than during fat formation, and suggest that investigations as to the relationship of metabolizable energy and the biological value of the proteins formed would be of value.

Mineral metabolism

During the past few years stress has been laid on the nutritive value of green food, conserved during the summer months, in the feeding of ruminants. Of the various methods of conservation perhaps the making of acid silage has been stressed more than others. In another section the effect of acid ensiling on the nutritive value of the grass proteins has been dealt with; in the present section a review of work carried out on the effect on mineral metabolism is given.

Brouwer and Dijkstra (33, 34, 35, 36, 37) has worked intensively on the effect of acid silage on the acid-base balance in cows. They found (33) that neither the butyric acid type of silage, fed at 20–30 kg. per day, nor the lactic acid type, fed at 20 kg. per day, produced an acidosis. He concluded that the organic acids were completely oxidized. An excess of base in the diet of a healthy cow was also tolerated with no obvious disturbance (34). It can be concluded, therefore, that the addition of $CaCO_3$ or Na_2CO_3 to a mineral acid silage would not be harmful to the animals. The feeding of large amounts of acid silage, on the other hand, was accompanied in some cases by a fall in appetite and a staring coat (35). The general symptoms were, however, a fall in the *p*H and alkali reserve of the blood plasma and a rise in the output of urinary ammonia. These symptoms could be prevented by the addition of $NaHCO_3$ to the silage before feeding. In a later experiment (36) the effect on the acid-base balance of butyric acid silage was compared with molasses silage and then with hay. In the first and third periods 20 and 30 kg. of butyric acid silage were fed daily with a daily organic acid intake of 392–535 and 676 g. respectively. In the second period 20 kg. molasses silage were fed with a daily organic acid intake of 356–433 g. The *p*H of the urine was 0·1–0·4 lower in the silage periods than with the hay, but in all cases the urine was alkaline. No change was found in the *p*H or CO_2 content of the blood plasma. The effect of the addition of large amounts of minerals to a silage ration yielded interesting results (37). To a ration of hay, grass silage and concentrates were added $NaHCO_3$, $KHCO_3$, $MgCO_3$ and $Ca_3(PO_4)_2$ so that the ratio of Na : K : Ca : Mg : P was unchanged. When the base reached a level of intake of 900 g. per head per day the *p*H of the urine rose from 8·10 to 8·27, the CO_2 content from 236 to 555 vol. per cent, while the organic acids fell from 123 to 52 mg. equivalents per litre. The last reduction was clearly the result of an increase in urinary volume. The CO_2 content of the blood plasma rose with a small addition of base from 61·4 to 67·9 vol. per cent, and was only slightly affected by a large increase in base intake.

In a comparison of ordinary clover silage and clover silage made by the Defu process, Gramatzki (38) found both types well digested. With the acid (Defu) silage more calcium was found in the urine, and when feeding the silage alone a negative calcium balance resulted, although the phosphorus metabolism was undisturbed. He suggests that acid silage should not be fed more than 50 per cent of the dry matter of the ration. Kirsch (39) obtained similar results, while in a later paper (40) he showed that both the calcium and phosphorus metabolism were adversely affected by large amounts of mineral acids.

The effect of carbohydrate foods on the calcium balance has been investigated (41). With a dry cow, in which only one-fortieth of the excreted calcium was found in the urine, a negative calcium balance was markedly improved by the addition of 200 g.

sugar to the diet. With a lactating cow, giving 5 litres of milk daily, a negative calcium balance was unaffected by sugar but was improved by wheat bran. The effect of lactation on the phosphorus requirements of dairy cows is shown in the experiments of Lamb *et al.*(42). With heifers, before calving, a positive phosphorus balance was noted with an intake of 11·6 g. P daily. During lactation this amount gave a negative balance which could be improved by the addition of bone meal to the ration. Bone meal is also an effective source of calcium(43). In a diet high in protein, energy and phosphorus, but low in calcium, the addition of bone meal as 2 per cent of the ration gave an increase in milk yield with twelve Jersey cows together with more persistent production throughout longer lactation periods and a higher degree of mineral storage as shown by the increased breaking strength of the bones. Comparing lucerne and timothy hay Holdaway *et al.*(44) found that with lucerne the body gain of calcium was greater than with timothy, becoming more marked as the lactation advanced. The phosphorus retention varied, being greater in the fourth to ninth month of lactation than in the first to fourth month. Forbes(45) in a detailed study of twelve cows over 1 year suggested that cows only need mineral supplements when the rations were abnormally poor in calcium or phosphorus, or when little roughage was given. This conclusion was also reached by Meigs *et al.*(46). In a feeding experiment over 6 years on diets high and low in calcium, in which calcium balances, bone analyses and carcase analyses were carried out, they found that the percentages of Ca and P in the fat-free bodies of mature cows and the Ca : P ratio in the bone and body remained constant irrespective of the calcium intake. They suggest that failure in reproduction in experiments by other workers was due to a deficiency of vitamin A. Groenewald(47) compared the blood analyses of cows and calves and the milk analyses of cows on rations deficient in one or more minerals. Phosphorus was the only mineral which showed decrease in the blood with a decreased intake, falling from 7·2 to 3·4 mg. per 100 ml. blood. The milk composition remained unchanged throughout the experiment. In the calves the blood calcium was higher than for the dams for the first 3 months and the potassium very high at birth but reaching the adult level at 10 weeks. The sodium was the reverse of the potassium, being low at birth.

The possibility of retention of considerable amounts of calcium and phosphorus in the alimentary tract of cows for several weeks has been raised by Turner *et al.*(48). Carrying out balance experiments in which orthophosphoric acid, mono-, di-, trisodium phosphate were compared, they found that the balances were irregular. They suggested a retention in the alimentary tract which, if present, would be represented in a short balance experiment as a true positive tissue balance.

The requirements for calcium and phosphorus have been reviewed by Huffman(49) and Jones *et al.*(50). They found that for maintenance 10 g. Ca and 10 g. P were required per 1000 lb. live weight. For milk production the requirement was 0·5–1·0 g. Ca and P per lb. milk. Lintzel(51) agrees with these figures but calculates the mineral requirements per kilogram of starch equivalent, using good hay as a standard.

An interesting paper by Fairbanks & Mitchell(52) has recently appeared in which the calcium requirements of growing rats are discussed. The authors concluded that for maintenance the calcium requirement is negligible in the growing animal. If this holds for the ruminant the question of mineral requirements will have to be re-examined.

The effect of a phosphorus deficiency on the digestibility of a ration is shown in the experiments of Riddell *et al.*(53). Two cows were put on a phosphorus deficient diet until marked signs of an aphosphorosis were showing. Digestibility trials showed that the ration was as well digested by these two animals as by the control animal on a normal diet. The two cows had a negative balance of calcium and phosphorus, whereas the control had a positive balance. With the phosphorus-deficient ration a

much greater urinary calcium excretion was found, while the urinary phosphorus was very low. Maynard *et al.*(54), on the other hand, found that a phosphorus-deficient diet, consisting of wet beet pulp, beet molasses and alfalfa hay, gave marked signs of pica, a depressed appetite and a poor gain in weight. Addition of steamed bone meal, or cotton-seed cake supplied sufficient phosphorus to remedy the deficiency. With the added phosphorus an increase in the phosphorus content of the plasma took place.

The effect of iodine supplements on health, reproduction and milk yield has been examined by Thomson(55). Using ten controls and ten experimental cows over five lactations, no fresh green food being given in the ration, he found that iodine, at the rate of 90 mg. per head per day, seemed to maintain health and milk yield. It also appeared to have some effect in the prevention of contagious abortion. On the other hand, Ahreus(56), using sows, found no beneficial effect on health, breeding qualities or resistance to disease by the addition of 0·1 per cent iodine, as potassium iodide, to the ration.

In a study of the effects of a vitamin A-deficient diet on the health of cows, Meigs & Converse(57) found that an A-deficient diet (timothy hay) caused cows to throw premature weak or dead calves. In less than 3 years the cows either died or failed to become pregnant. Milk yield was reduced after the first year. The young calves, up to 6 months, were highly susceptible to vitamin A deficiency, but less likely to die after that period if one-half of the ration was hay of a high vitamin content. In a later paper(58) they suggest that the disorders of health and metabolism periodically found when feeding a low calcium ration are really due to a deficiency of vitamin A. The clinical symptoms of vitamin A deficiency in ruminants has been studied by Guilbert & Hinshaw(59). The ration consisted of dry molasses beet pulp, rolled barley, cotton-seed meal and calcium carbonate. In 240 days the vitamin A reserves of the body were exhausted, and in 282 days the animals were very ill. In the absence of infectious abortion a vitamin A deficiency produced premature expulsion of the foetus. Calves born from heifers which had been on a restricted vitamin A diet during gestation had severe diarrhoea at 2–8 days. The milk of the dam was deficient in vitamin A. Calves may also suffer from night blindness. Moore & Huffman(60) have recently noted a new type of vitamin A deficiency in young calves. Where poor quality roughage was fed a nutritional blindness, different from the night blindness of a vitamin A deficiency, was found. This was associated with paralysis, weakness and poor reproductive capacity. It was not connected with a calcium deficiency, and could be prevented by feeding corn silage, timothy hay or cod-liver oil.

Duncan & Huffman(61) studied the effect of a vitamin D-deficient ration on dairy calves. They found that a deficiency led to a progressive downward trend in plasma calcium and inorganic phosphorus, anorexia, stiffness and bowing of legs and a reduction of the ash of the ribs, and to a decrease in the growth rate.

Rationing

During the past 2 years much attention has been paid to the conservation of young grass, either by drying or by ensiling, and a great deal of work has been carried out by using the material as part ration for the lactating cow. In the previous review(62) brief reference was made to a few preliminary experiments in the rationing of dried and ensiled grass. More conclusive evidence as to the nutritive value of the product has since been published.

Camburn(63), using a short period trial with ten cows, replaced maize silage with dried grass. An increase in milk production and butterfat resulted. Camburn also stated that a loss in weight of the cows was noted with the dried-grass ration. The loss, however, amounted only to a few pounds, which, with ruminants, is insignificant,

unless weighings are carefully checked over a period of days with a large number of animals. Crichton(64) replaced 15 lb. swedes, 4 lb. straw and 4 lb. concentrates by 10 lb. dried grass, or 30 lb. silage by 8 lb. dried lucerne. An increase in milk yield and in butterfat colour was noted. Knott & Hodgson(65) concluded, from an experiment with two groups of four cows and two groups of two cows, that artificially dried grass may be used as part of the concentrate ration for lactating cows. Newlander(66) carried out digestibility trials with cows using artificially dried silage. The protein digestibility was lowered by 15 per cent. It is possible in this case that a high temperature was used in the drying process. In a later section it will be shown that the nutritive value and digestibility of the protein becomes seriously affected when a high temperature is used in drying.

Rehm(67) investigated the nutritive value of different types of silage. Lucerne was ensiled by the acid process and by storage in a silo with an airtight cover to prevent loss of CO_2. The latter method gave a lower wastage and a product with a higher digestibility and better influence on milk yield. Patterson(68) and Peterson *et al.*(69) found that A.I.V. silage was as good as an ordinary dairy ration, and also increased the carotene content of the milk. In the former experiment(68), however, little importance can be attached to the results since the silage (40 lb.) replaced 40 lb. mangolds and 5 lb. hay in the maintenance diet, the production ration remaining constant.

A series of experiments by Watson and his co-workers(70,71,72) and by Morris *et al.*(73) are interesting in that they compare artificially dried grass and various types of silage. In the first paper Watson & Horton(70) give tables showing the composition, digestibility, coefficients and digestible nutrients of fresh grass, hay, silage and dried grass. From curves given, the starch and protein equivalent can be found by interpolation if the value of the crude protein is known. In the second series of experiments Watson & Ferguson(71) compared dried grass with a concentrate ration, only a part of the concentrates being replaced by grass. Two groups of ten cows were used, made up of Guernseys, Ayrshires, Friesians and Shorthorns, each feeding period being for 5 weeks. The protein equivalent of the ration was varied from 0·49 to 0·60 lb. per 10 lb. milk. No difference in the two rations could be noted as regards either milk yield or butterfat. The increase in the carotene content of the milk was, however, very marked during the grass periods. In the third series(72) dried grass was compared with A.I.V. and molasses silage, using 20 cows, in experimental periods of about 4 weeks. The protein equivalent of the production ration was about 0·50 lb. per 10 lb. milk, and the conserved grass supplied about 50 per cent of the nitrogen of the production ration. No significant difference could be found between the types of conserved grass as regards either milk yield, amount of butterfat or weight of the animals.

Morris *et al.*(73) in a series of experiments found that fresh spring grass, dried spring grass and three types of silage, A.I.V., molasses and ordinary, were as good as dried blood and superior to bean meal when fed, along with oats and maize as the production ration, at a level of 0·45 lb. protein per 10 lb. milk. On the other hand, both fresh autumn grass and dried autumn grass, cut at the end of September and beginning of October, were markedly inferior in nutritive value to the spring grass either fresh or conserved.

From the complete set of results of all workers it can be safely concluded that conserved grass, if cut and conserved before September, is as good for milk production and general health as the best concentrate ration even when the grass is fed as *part* of the production ration at a level in which the whole production ration supplies only 0·45 lb. protein per 10 lb. milk.

Regarding the nutritive value of skim milk contradictory results have been

published. Whereas Reed(74) and Woodward *et al.*(75) found that it was unpalatable and gave a fall in milk yield, Pirrochi *et al.*(76) found very favourable results when comparing skim milk with linseed cake. An increase in milk yield and an increase in the weight of the cows was noted during the skim-milk periods.

A new concentrate food, wood-sugar yeast, with a starch equivalent of 66·7 and a digestible protein content of 37·6 per cent has been used in Germany(77, 78, 79, 80). The results appear to show that wood-sugar yeast is as good as soya-bean meal for milk production.

A review of the nutritive value of dried blood has recently appeared(81). The author gives a résumé of work up to date together with his own feeding trials. It is clear that dried blood, when dried at a low temperature, is a very valuable food for the nutrition of farm animals.

Investigating the nutritive value of soya-bean cake, Takahashi *et al.*(82) found a progressive increase in milk yield as the intake was increased from 20 to 50 per cent of the production ration. They conclude, however, that, due to the high fat content of the cake, it is safer to feed only 35 per cent of the ration as soya bean and add cotton-seed meal. Comparing calves on soya-bean flour and milk, Shoptaw(83) noted that the former gave a smaller increase in height and weight than the latter, although the animals ate more grain and less alfalfa hay than those fed on milk. Generally, however, the soya-fed calves lacked the thrifty condition of the milk-fed group.

With herring meal, Schmidt *et al.*(84) found that an increased milk yield resulted when it replaced an equivalent amount of protein in a soya cake-rye mixture. Fish and kelp meal(85) had no beneficial effect when replacing a good dairy ration, whereas a grain mixture containing 8 per cent manhaden fish meal was better for reproduction and health than a ration with linseed and cotton-seed meals(86). With the fish meal there were fewer cases of retained afterbirth, fewer breedings were necessary and the birth weight of the calves was higher. Lush(87) noted an increase in milk yield when rations containing 10–19·4 per cent shrimp meal replaced a cotton-seed meal diet. No abnormal flavour was found in the milk. Comparisons of tankage with a mixture of soya-bean meal and cotton-seed meal(88) and pea meal with linseed meal(89) showed no extra benefit in the feeding of any of the rations. In the pea-meal experiment(89) it is obvious that no difference could possibly be found since alfalfa hay and sunflower silage were fed with both rations, yielding a large surplus over the actual requirements for milk production.

Interesting publications by Atkeson(90) and White(91) show that, whereas when feeding succulent foods a decrease in water intake was noted, the total water intake remained constant. Cows giving 8 gal. of milk drank 19 gal. of water.

The type of concentrate to be fed to cows when at pasture has been studied by Perkins(92) and Horwood *et al.*(93). Both experiments show that under ordinary pasture conditions a ration containing 16 per cent protein gives an increase in milk yield over one containing 9–11 per cent protein. The increase in milk was, however, uneconomical.

The effect of protein level has been studied by Harrison *et al.*(94) and Henke & Goo(95). Using three groups of twelve cows on 16, 20 and 24 per cent protein rations, Harrison(94) found that the 20 per cent ration gave a slightly better yield of milk than the 16 per cent ration. Henke & Goo(95), on the other hand, found that yield of milk remained unchanged with rations containing either 10·8 or 20·2 per cent digestible crude protein. It is clear that, unless the quality of the protein fed is known and taken into account when rationing, results may vary markedly, and can only lead to further confusion in any system of rationing.

Examining the influence of fat intake on milk yield and butterfat production, Moczarski & Bormann(96) found that, when the fat in the ration was less than 1 part,

per 1000 parts live weight, there was a fall in milk yield and butterfat. McCandlish & Struthers (97) fed 1 lb. butterfat per head per day to lactating cows but no increase in either milk yield or butterfat resulted. Animals tended to lose their appetite on adding fat to the ration. On changing cows from stall to pasture feeding, Johansson (98) found a rise in the percentage of butterfat. This could be explained partially by a fall in milk yield, but some other factor also seemed to be present, affecting butterfat production.

An interesting paper by Leroy (99) has recently appeared. He gives an exponential equation showing the normal fall in milk yield from 4 to 6 weeks after calving. Any marked deviation from this equation, he claims, shows that an inadequate ration is being fed. A table is also given showing the expected milk yield, and the adequacy of a diet can be found by comparing the actual milk yield with that of the table. If the yield is lower this can be increased, according to Leroy, by supplementing the ration.

Protein metabolism

(*a*) *The amino acids in nutrition*

Continuing the work previously reviewed (62), Morris & Wright (100) studied the amino-acid requirements of ruminants for maintenance of body weight. They found that, whereas lysine is essential, cystine, if necessary, need only be supplied in small amounts if sulphur in some other form is given in adequate amounts. These results differ from those obtained in experiments with sheep (101, 102) in which cystine was found to be essential for wool growth. It must, however, be stressed that the maintenance requirements of cystine are small and could be synthesized by the rumen bacteria, whereas for wool growth the requirements are very large and synthesis of the total amount necessary probably impossible.

Recently Womack and Rose (103) have investigated the indispensability of leucine and iso-leucine for growth and maintenance of rats. They found that both were essential. Continuing their research into the nature of the unknown essential amino acid Rose *et al.* (104, 105) found that this was β-amino-hydroxybutyric acid.

During the past 2 years much attention has been drawn to the effect of high temperatures on the nutritive value of proteins (106, 107, 108, 109, 110, 111, 112). Whereas the majority of workers found that a high temperature lowered markedly the biological value, a depression which could be relieved by the addition of lysine to the diet, Hayward *et al.* (111) noted an increase in the nutritive value and digestibility of soya beans on heating to 121° C. for 90 min. In view of the mass of contradictory evidence further confirmation of the work of Hayward seems essential. Block *et al.* (112) analysed proteins before and after high-temperature treatment and found no change in the lysine content. He suggested that the lysine precursor in the protein molecule has been rendered indigestible by the heat treatment. The importance of this subject, with respect to the preparation of foods for dairy stock, cannot be denied owing to the methods at present in use for the drying of such important foodstuffs as blood meal and grass.

Recently Miller (113) published a series of results on the amino acids of grass proteins using the Block modification (114) of the Vickery method (115, 116). This consists of the isolation and gravimetric determination of the diamino acids, arginine, histidine and lysine, the two former as the flavianates and the latter as the picrate. The results obtained by Miller (113) are markedly lower than those found by Chibnall (117, 118, 119) who used the Van Slyke technique (120). It is obvious that further work is essential to determine which if either of the two methods is more exact, and whether an alternative or a modified method can be devised which will give more exact results. Especial care must, however, be taken in the calculation of the lysine content

of any ration that the same method is used for each foodstuff composing the ration.

Virtanen and Laine (121) have recently published a modified technique for the colorimetric estimation of tryptophane in grasses. According to the authors the method is accurate to within 5 per cent. The results are very much lower than those found by other workers using the insoluble humin fraction obtained by acid hydrolysis as an estimate of the tryptophane (117, 118, 119). Some interesting changes in the tryptophane content of the plant with growth have been noted.

(*b*) *Non-protein nitrogen*

Ehrenberg & Scholz (122) found that ammonium bicarbonate and acid silage could replace for the great part an oil-cake concentrate at less cost and with only a small diminution in milk yield. To replace 4·5 kg. oil cake they fed 1 kg. oil cake, 13 kg. acid silage, 0·819 kg. NH_4HCO_3 and 1 kg. molasses. With complete replacement a loss in body weight and a diminution in milk yield resulted. In view of this fact it seems possible that the bicarbonate merely replaced non-essential nitrogen, the essential amino acids being supplied by the silage and oil cake. In a later publication Ehrenberg *et al.* (123) draw attention to the fact that in evaluating non-protein nitrogen the amino-acid make-up of the protein fed concurrently must be known. It is clear that the evaluation of the non-protein nitrogen fraction of any ration, such as silage, is of little assistance in the determination of the nutritive value of non-protein nitrogen unless the exact composition of the fraction is known. If, as in silage, the fraction consists of amino acids which originally built up the silage protein, then the nutritive value of non-protein nitrogen cannot differ markedly from that of the original protein, depending on the amino acids which have been split off. Thus the results of Watson & Ferguson (72) which show the high value of the non-protein fraction of the silage are of little help in considering the nutritive value of non-protein nitrogen.

(*c*) *Nitrogen metabolism*

In any attempt at an understanding of the nitrogen metabolism of an animal a knowledge of the endogenous catabolism is essential. Recently Hutchinson & Morris (124, 125) investigated the endogenous metabolism of goats, sheep and cows, together with the fasting catabolism. The results confirm those of Schneider (126, 127) and Mitchell (128) that faecal nitrogen is proportional to the dry food intake. With a decreased intake the ratio of faecal nitrogen to food intake rose markedly owing to the importance of the constant fraction, i.e. the fraction excreted during starvation. A study of the urinary partition products showed that during both catabolism (starvation) and anabolism (heavy feeding following starvation) the creatine metabolism is markedly increased. On the other hand, during deamination only, no increase was found. It was found that the difference between the endogenous and fasting catabolism could be explained by a protein metabolism similar to that found on ingestion of protein.

In a study of the digestibility of proteins (125) the authors found that digestibility varied with the protein requirements of the animals, the greater the requirement the greater the digestibility.

During the maintenance experiments (100), as in those during lactation (62) with a decrease in the biological value, an increase in ammonia and creatine output was noted together with a nitrogen : sulphur ratio of the excess output closely approaching that of body tissue. This showed, once again, that tissue catabolism takes place during the feeding of a deficient ration.

Regarding the origin of the blood ammonia, experiments with sheep (129) have

shown that there are in sheep's blood two substances whose spontaneous breakdown *in vitro* give ammonia. One of these substances is identical with adenylic acid.

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III. GENETICS

General

Von Patow's "byre average" was discussed at some length in the last Biennial Review. The system aims at assessing the true genetic value of a lactation yield by considering it in relation to the average production of all the animals in the byre during the period at which the yield was made. The practice rests on the assumption that differences between herd averages and between annual averages are entirely matters of environment and management in contradistinction to the practice of using actual records without paying attention to the herd or year in which they were made, which infers that differences in herd or year averages are entirely genetic.

Lush (1), in a critical article surveying the work of the Von Patow School, crystallizes the thoughts of many investigators in stating that the truth is somewhere in between. He believes that it is unlikely that the Von Patow method can ever lead to a Mendelian analysis which corresponds at all to reality, except in so far as both it and the reality can be described at least roughly by something like a normal curve. He adds that the expression of yields as a percentage of the average yield of a large herd automatically gives a distribution of figures which average not far from 100, and has somewhere near a normal distribution: the demonstration that such a distribution can be described, at least roughly, by the curve of $p+q$ to the sixth power, where p and q each equal $\frac{1}{2}$, seems to prove very little. This, maintains Lush, is a hypothesis that $p+q$ will always equal $\frac{1}{2}$ if the herd is large enough. What is the value of excluding "abnormal" lactations? In actual practice the method leads to the same result as a sire index capable of dividing the population into only seven classes from highest to lowest producers, except that more weight is given here than in most indexes to the results of mating sires with extremely high, or extremely low, producers, and correspondingly less attention is given to the results of matings with average females.

Another aspect of the problem has been stated by Gowen (2) in a study based on the records of some 14,000 Jersey cows comprised in the Register of Merit of the American Jersey Cattle Club which completed their lactations between 1900 and 1923. A cow's whole inheritance for milk production is ascertained by comparing her production with that of her parents, sisters, cousins, and grandparents. Environmental factors are determined by comparing the production of individuals of the same generation within the same herd with that of other generations. Correlations between the different relationship groups are as follows:

Relationship	No. of pairs	Milk yield
Same sire: dams by same bull	14,426	0·31
Same sire: dams unrelated	99,458	0·24
Same paternal grandsire	489,526	0·02
Same paternal granddam	26,028	0·06
Same maternal grandsire	64,150	0·12
Same maternal granddam	3,198	0·15

After discussing these figures Gowen gives values for measuring the effect of dominance and of variance in heredity and environment. He interprets his figures as implying, for both milk yield and butterfat percentage, that the presence of genetic factors without dominance variations in heredity account for about half of the observed variation in milk yield, and four-fifths of the observed variation in butterfat percentage. Thus he concludes that environment plays but a small part. As regards the possibility of any of the factors governing the inheritance of milk yield being transmitted in a sex-linked manner, he confirms previous workers that butterfat is largely autosomal. As regards milk yield he states that the data presented do not support the sex-linked hypothesis. It is not easy to follow this statement. The suggestion that sex-linked characters exist depends upon a low correlation to the paternal grandsire as compared to that of the maternal grandsire. In the figures presented by the author the numbers are adequate, that to the common paternal grandsire consisting of 489,526 pairs, and that to the maternal grandsire of 64,150 pairs. As regards milk yield the correlation to the paternal grandsire is 0·02 and to the maternal grandsire 0·12: in other words, the correlation to the maternal grandsire is six times greater than to the paternal grandsire.

Plum (3) has also dealt with the question of heredity and environment, and has based his observations on the Iowa Cow Testing Associations. Besides an interesting review on the subject, he deals solely with his own data as butterfat production measured in pounds. (Incidentally it would appear to be becoming generally recog-

nized that it is not reasonable to trace the inheritance of percentage butterfat.) The figures were analysed by Fisher's method of analysis of variance. After the variance between the classes had been isolated there was a residual variance, and between this and the total variance a correlation was made which showed the variance affecting all members of the same class. Plum concludes that, when pure-bred and grade cows are compared, breed differences account for only 2 per cent of the total variance, but pure-bred pedigree cows show distinctly greater variance between breeds than do grade cattle. Greater variance was found between different herds. This amounted to 34 per cent less than the interbreed variance. The effect may be due to several causes: each herd may be a distinct group with a certain uniformity obtained either by selection or relationship of animals to each other; alternatively the environment and management may be uniform in one herd, but different from herd to herd. This figure of 33 per cent due to herd differences is probably correct, and confirms previous work by Gowen. As regards variation due to differences between cows, 40 per cent of the variance inside a herd, and 60 per cent of the variation between herds is due to differences in yields between cows. Accordingly breed, herd, and cow differences account for 61 per cent of the total variance: the remaining 39 per cent is due to differences between the records of the same cow. Such differences may be caused by a number of factors, such as year to year changes in weather, management and feeding, length of preceding dry period, season of calving, etc.: these are discussed in greater detail.

The estimate of the genetic part of the variance obtained by Plum is somewhat lower than the values given by Gowen, who reckon that in butterfat production the hereditary effect is to be calculated at some 75–80 per cent. Another conclusion of some genetical importance is that the genetic part of the variance, which goes from one generation to another, is approximately one-quarter. The practical consequence of this is that if cows, selected as parents for the next generation, average 100 lb. butterfat above the level of the breed or herd, and if the bulls are equally superior, then the progeny would average about 25 lb. above this same level. If the average interval between generations is 5 years, this would correspond to an improvement of 5 lb. of butterfat per year. Looking at the problem from year to year the rate of improvement is slow, but from generation to generation, which is the true measure, it is substantial. Incidentally, the rate is much the same as that arrived at by Lush and quoted in detail in the last Biennial Review.

Krizenecky(4) has found a high positive correlation between milk yield and fat yield. In a later paper Krizenecky & Lantuskin(5) discuss the matter further, confirming the previous conclusions that the curve for absolute fat production follows that for milk yield in parabola.

Butterfat and other constituents

Whetham & Hammond(6), in an investigation based on the London Dairy Show records for the years 1923–33, state that the reason why certain sires can effect an improvement in milk yield in conjunction with, or independently of, fat yield, is affected by an increase in the number of cells, in which case fat yields are also increased, although the fat percentage remains the same, or by an increase in the rate of milk secretion, in which case the fat percentage is reduced. They suggest that the size of the fat globule is determined by two factors, the rate of butterfat formation by the cell, which varies with breed and stage of lactation, and by the rate of milk secretion which affects the size of the globules by the rate at which they are washed out from the cell.

A paper by Allen(7), who found that the butterfat content of milk could be appreciably increased for short periods of time by feeding grain rations enriched with

fat, has created a considerable discussion on this subject. Garner & Sanders(8) report similar results from butter and palm oil. M'Candlish & Struthers(9) found that when cows were fed 1 lb. of butterfat per head per day either in the form of butter or of cream, there was no significant effect on the yield or composition of the milk. The animals had a tendency to go off their feed at the beginning and end of the fat-feeding periods, which were of 10 and 6 days' duration. McCay & Maynard(10) fed cod-liver oil, shark-liver oil, and salmon oil for periods of 2 weeks with intervening periods without oil. Cod-liver oil lowered the fat percentage, but shark-liver oil and salmon oil had no effect. All oils caused a rise in the iodine number of the milk fat. The results, which are discussed in detail, indicate that cod-liver oil exerts its effect directly on the mammary gland rather than by indirect alterations in the composition of the blood.

Whetham & Hammond(11), again working with the results of yields of cows at the London Dairy Show, measured the colour of the milk, and analysed the results statistically. They conclude that the two most important factors affecting butter colour are the genetic character of the cow and the method of feeding. A slight increase in the shade of colour was found to occur under most of the various conditions which give rise to increased milk yield. The colour is high in the first few weeks of lactation, and falls gradually as more fat is required from the body in addition to that from the food. After about 180 days the colour rises again as the butter yield falls, and more of the fat is supplied by food intake. It is suggested that butterfat derived from body fat will be paler than from food fat.

Gowen & Tobey(12) conducted an experiment on cows receiving no food, but having access to all the water they desired. All the changes in milk composition during starvation could be directly related to the simultaneous changes in the blood. The same workers(13) discuss this subject further, as well as the influence of insulin and phloridzin. The fact that the different constituents of the milk did not exactly parallel each other in their behaviour throughout the various experiments leads the authors to conclude that they have, in all probability, separate origins. The wide variation brought about in the constituents points to the conclusion that in milk secretion a balance is maintained between osmotic pressure of the milk and of the blood. These experiments give direct proof for the conclusion that modifications of the blood of dairy cattle produce direct and predictable modification of the milk secreted.

In the past it has been taken for granted that lactose is the least variable of the major constituents of milk. A review of the literature by Brown *et al.*(14) reveals a lack of experimental data in support of this. Accordingly these authors studied the relationship of blood sugar and lactose in pedigree Jerseys and Guernseys with free access to water. Difficulties in the technique of the method are discussed.

If the minimum protein requirements for milk are covered, will a further increase in the amount of protein in the ration have any stimulative effect on milk production? Harrison *et al.*(15) found that cows on 20 per cent total protein produced a little more milk, but this increase is not sufficient to warrant the cost of the 20 per cent ration. High planes of protein intake had no stimulative effect on milk flow.

An analysis of the vitamin A value by Hilton *et al.*(16) again emphasizes that nutrition is the dominating factor in respect of the secretion of this vitamin in the milk. Beeson(17) made a similar study with similar conclusions. Kon & Henry(18), dealing with the vitamin D content of Guernsey and Shorthorn butterfats, state that the differences in the vitamin D composition of the two types of milk could be simply expressed by the ratio of the fat percentages.

Groenewald(19) has studied the mineral composition of the blood and milk of cows, and of the blood of their progeny with eleven groups, consisting of two graded Friesland (South Africa) heifers. Phosphorus was the only mineral of which a shortage in

the ration was reflected in the composition of the blood of the heifers, but this was not followed by a decrease in the amount secreted in the milk. Variations in the composition of the rations had no influence on the composition of the milk, the minerals studied being calcium, inorganic phosphorus, total phosphorus, magnesium, potash, sodium, and chlorine. Sharp & Struble(20) have studied the chloride value of milk as determined by direct titration with silver nitrate. A distinct breed difference was observed, Holsteins having a higher value than Guernseys and Jerseys: the value is lower with advancing gestation.

Using a modification of the Hill technique, a study was made by Riddell *et al.*(21) of the principal factors influencing the curd tension of milk under normal conditions. Future workers on the genetic aspect of this subject should refer to this paper. The authors confirm previous work, that the breed was found to be one of the most important factors influencing curd tension: they rank the breeds in the following order of decreasing importance as producers of soft-curd milk—Holstein, Ayrshire, Guernsey, and Jersey.

Guthrie & Brueckner(22) found no genetic connexion with the occurrence of "oxidized" flavours in the milk.

Conformation

There has been renewed activity on the question of conformation in relation to milk production. Several workers on the Continent of Europe claim to have established definite correlations. This is not in accord with existing knowledge. The following are a selection of recent papers on the subject: Gowen(23) dealt with the correlation coefficients between related animals of the Jersey breed in connexion with their body type and conformation. He concludes that these correlations show that inheritance accounts for most of the variation in the size of the cattle, and that such environmental differences as do exist play but a small part in their ultimate constitution. Cristea(24), working with the Swiss Simmentals from seven herds, and measuring the rib angle by Duerst's goniometer, found a definite correlation between the rib angle and milk production, and concludes that, by this method, it is possible to determine the approximate productivity of an animal, as well as the constitutional type. Schuppli(25) emphasizes the importance of "formalism" in dairy cattle, and states that, for their proper judging for production, it is necessary to take a number of measurements, such as height at withers and chest, width and depth, rump width, and body length. He claims that a good correlation can be obtained between body conformation and milk production. Another series of measurements has been made by Wömpner(26) on various breeds of German and Austrian cattle. He claims to have obtained a significant positive correlation between nasal length and performance; other relationships between the head and milk production are also given, as well as diameter of the horns. He asserts that head measurements are of definite practical importance to the progressive breeder. Borgioli(27), dealing with Brown Swiss cattle in Italy from twelve different herds, found fairly high positive correlations with milk yield in respect of body weight, surface area of hind quarters, chest girth, and head length. A negative correlation of $-0{\cdot}25$ was found between tail thickness and milk yield. This author, however, warns against relying on conformation as the sole basis of selection. Feuersanger(28) deals with the importance of skin and hair as a means of estimating milking capacity. The skin is judged by its softness and its thickness, and the hair by its strength and lustre. Personal skill in judging by sight, and more particularly by touch, is more reliable than measurements, which are never free from considerable sources of error. While the lustre of the coat is important in judging the general state of health, he concludes that it is not particularly useful in estimating milk yield.

In contrast to these observations are those of Kroon (29) who discusses the situation generally, with special reference to Dutch cattle. He states that the earlier views on external appearance as a clue to production have been gradually abandoned as it was realized that correlation between structure and function is not as close as it was at first thought to be. He found that analysis of blood did not reveal any correlation with production. In discussing the relative merits of judging by type and by performance he concludes that the former cannot be dispensed with altogether, though records of performance provide a better basis for judging performance ability. Type is more useful as a measure for constitution, predisposition, or beauty of the animal. Secondary sexual characteristics should be well developed, since this indicates active endocrine functioning and, consequently, presumably high yield of cows and breeding worth of both sires and dams. Kovalevskiĭ (30) deprecates the modern method of progeny testing as being slow and laborious, and sets out to discover a "short cut". Early maturity is associated with the large cell size, high water content of tissues, and a greater development of connective tissue, and high yielding cows are characterized by the large size of cells of the secretory epithelium. He states that the investigations of Ivanova also established a correlation between milk yield and erythrocyte size, and that this criterion may be introduced as a means for evaluating dairy sires. By the examination of 15–20 daughters by this method a degree of accuracy is obtained equal to a comparison of the yields of 400 daughters. A note of warning is sounded by the editor of the publication in which this paper appears, that further study is required before selection is made on the basis of cell size.

Efficiency of production

Some further work has been done on the efficiency of dairy cattle based on Gaines's 4 per cent milk. Brody & Ragsdale (31) have produced tables whereby milk containing any fat percentage may be easily converted to milk containing 4 per cent fat in order that the different yields may be placed on a comparable basis. They also draw certain conclusions from the different breeds of cattle at the Missouri Experiment Station. A large cow, to make up for her greater maintenance cost, must produce more milk than a small cow. If the large cow's extra milk is enough to pay for her extra maintenance cost, then both have the same nutritional efficiency. They state that the small cow tends to be slightly more efficient in the use of feed. In other words, the large cow's extra milk does not quite pay for her extra food consumption. This, however, is balanced by the greater overhead cost involved in keeping a larger number of smaller animals. The authors conclude by stating that they believe that udder capacity and stimuli to high milk production are more important than size in profitable milk production.

Edwards (32) has applied this formula to the records of 2400 cows at the London Dairy Show between 1922 and 1934. He finds that among the best representatives of the various dairy breeds there is little difference in gross efficiency of milk production, and that the lactation stimulus has been bred approximately in proportion to the size of the breed. In spite of their greater weight, cows are more efficient than heifers, and within the breed there is a slight tendency for gross efficiency to decrease with increase in live weight, but greater than any of the foregoing differences in gross efficiency is that which exists within a breed, due to differences in actual milk yield. In animals milked thrice daily the variation in efficiency was, for breeds, from 37 to 42·5 per cent. In one breed the individual range was from 34·5 to 44·5 per cent. It should be noted that both these studies are based on the ratio of the fat in the milk to the nutrients *theoretically* required for its production and the maintenance of the cow: they do not take into account the idiosyncracies of the cow herself as regards food consumption in relation to milk production.

Pedigree, breed construction, etc.

By far and away the most interesting experimental work which has occurred during the period under review is to be found in the *American Year Book of Agriculture* for 1936(33). Mr H. A. Wallace, Secretary of the Department of Agriculture, in his introduction, states that the principal function of his department is scientific research, and that all other activities are merely the practical expression of research results (p. 82). Indeed, the whole volume, which runs to 1189 pages, and deals almost entirely with genetic research in crops and animals, arises from an investigation begun 3 years ago by the Bureau of Dairy Industry. It was undoubtedly a unique, as well as a tremendous, undertaking to survey some 26,000,000 dairy cattle in order to determine what progress has been made in breeding for consistently good production, and to discover the best germplasm for producing ability. The study was restricted to herds in which records of production for a considerable percentage of the animals had been continuously maintained for a period of 8 years. The surveys actually made cover 1100 commercial, college, and experimental station herds. Of these 707 are analysed in the Year Book, representing 4302 sires and 29,570 tested daughters out of tested dams. The average butterfat yield of the entire group of daughters was 451 lb. as compared with 452 lb. for their dams. To determine the progress made in each herd the approximate ability of transmission of each sire was analysed. The sires were judged by three criteria, the improvement in production of the daughters over their dams, the proportion of daughters equal to, or better than, their dams, and thirdly, the relation of the production of the dams to the herd average. Briefly, the practical results of the investigation are below. (Though progeny testing proper is dealt with in a later section of this review, it is inconvenient to dissociate it from the other results of this investigation.)

Discussing the question of a sire with an inheritance for level of production below that of the herd, the writers cite various instances, and state that the answer is different for different sires and different dams. If the dams have a concentration of germplasm for the level expressed by their records, it is not likely that one cross of a sire whose inheritance is considerably lower will send the production to very low levels. But more than one cross of such a sire would probably bring about a much lower level. If a single poor sire is followed by a sire with a high inheritance, the production may again approach its former level, though it will probably take more than one cross of such sires to eliminate from the germplasm any low levels of production that the poor sire injected. In the survey it was apparent that in many herds the cows with the best records in each generation were kept as the brood cows for the next generation of females, the poorer producers being culled.

Again specific examples are cited which indicate that, even though the production level is maintained by the constant selection of the higher producing cows, there will be but little diminution in the number of cows with poor germplasm in the later generations, and there will be a continual need for culling. If the sires used possess an inheritance markedly lower than the dams, then the proportion of cows with poor germplasm will increase rapidly, despite the selection of the higher producers. In the experimental herds of the Bureau of Dairy Industry it has been the practice to keep every cow in the herd till she produces a heifer, and to test every cow under as nearly equal environmental conditions as possible, regardless of her producing capacity. Dealing particularly with the records from the Bureau herd at Huntley, Montana, it appears that progress can be made without culling in the female line if sires with the right kind of germplasm are used. If, on the other hand, poor sires are used, and there is no selection of brood cows, the figures indicate that the level of production sinks very rapidly to that governed by the inheritance possessed by the sires. Sires were

classed as poor when the average production of their daughters was less than that of the dams by a considerable margin, and when less than half the daughters were better than their dams. This was modified in the case where sires showed higher production than the herd average.

There is a discussion about the various types of poor sires, many of which were used, or bred, at the Huntley herd in co-operation with breeders for the testing of the bulls. The information on these different sires is well set out, and of the greatest interest. The writers emphasize that it is easy to underestimate the real value of a sire that is mated to dams of a very high level of production when his daughters do not come up to that level unless he has also been tried on dams of a lower level of production. One instance is of a bull who, in the Huntley herd, out of cows producing 1788 gallons, left ten daughters giving 1892 gallons. The same sire, used in three Nebraska herds, left daughters producing about 200 gallons less than their dams. Reckoned in butterfat, these daughters produced about 50 lb. less fat than did their dams, whereas at Huntley they produced 15 lb. more than their dams. The writers offer two possible explanations. The dams in the Nebraska herds probably had a higher level of production than the inheritance of the bull came up to. Difference in environmental conditions may have influenced the results to some extent, but it is probable that the dams in the Nebraska herds were not homozygous for as high a level of production as was indicated by their records, and that on the average they transmitted, in these matings, a lower level of production than the average of their actual yields. On the other hand, the dams in the Huntley Station herd may have been fairly homozygous for the level of milk yield indicated by their records.

It is maintained that there is every reason to believe that the inheritance governing the level of production is received from both parents. If, however, one parent were pure in its inheritance for the factors that would determine a high level of production, and the other parent were pure for the factors determining a low level of production, the parent with the high level inheritance might appear to have more influence on the producing capacity of the daughter than the parent with the low level inheritance. If this theory holds true—that fair, good, or excellent sires within a breed have the ability to transmit higher levels of production than are expressed by their dams—then, with each succeeding cross of such sires, the females of the herd would carry in their inheritance more factors that determine higher levels of production. Hence, though it is always desirable to know the good breeding dams, it would be more important to know them in a herd where only mediocre sires had been used.

While acknowledging the part played by superior breeding dams in the construction of the breeds, it is stated that greater emphasis has been put on the proven sire because the inheritance he possesses can be determined at an earlier stage than is the case with the female, and also because his inheritance is passed to perhaps all of the next generation in a given herd. An examination of the production records through the female lines of descent with two or more successive crosses of proven sires shows how quickly the identity of the foundation cow with a high butterfat yield is lost in her descendants under the levelling of the inheritance introduced through two or three sires.

The selection of a young bull that has the greatest probability of possessing an inheritance with a considerable concentration of factors that determine high levels of production demands that both his sire and his dam should have such a high concentration. A note of warning is sounded against advising farmers to use proven sires, because there are so few proven good sires available. However, the opinion is stated that if nothing but sires whose good merit had been definitely proved were used in a given herd, that herd would finally carry many of the factors for determining high levels of production. Figures are given of the transmitting ability of bull calves, the

sons of proven sires, only three of which failed to increase the average yield of butterfat. The conclusion is reached that the sons of sires of proved merit do possess the ability to increase the level of production in the herds in which they are used.

There is an imposing appendix, which cites the herds which have co-operated in this work, and which summarizes the work accomplished in a number of these herds, and gives a list of workers who are associated in the investigation.

In addition to a general article on "The Genetics of Cattle", there is a summary of the work on inbreeding which has been conducted at the United States Department of Agriculture Farms. This was discussed in the previous review, but the additional figures are interesting.

A paper by Regan *et al.* (34) on 18 years' close inbreeding with the Jersey herd at the New Jersey Experiment Station is worth studying. In the spring of 1918 the herd consisted of three bulls and twenty-two females. Four years later twenty-nine Jerseys were added, but since then no outside blood has entered the herd, and sire to daughter matings are practised. When necessary to replace a bull, his most highly inbred son, out of a cow possessing desirable type and production, was selected to take his place. The results of 197 dam-daughter comparisons shows an average increase for the daughters of 46 lb. of fat. The daughters of every bull have averaged better than their dams. There has been no loss in body size or breeding efficiency, but certain recessive defects have been noticed, such as hairlessness, congenital blindness, and a form of achrondroplasia.

There are a considerable number of studies based primarily on the records of Milk Recording Societies, and of these the following are of interest: Varecka (35), dealing with the cattle breeds of Czechoslovakia, confirms the previous work of Krizenecky, and states that the increase of fat production goes parallel with an increase of fat yield. He states that, in order to improve the cattle of his country, it is essential to develop milk recording and to record all animals in a byre. He makes the curious statement that fat recording will not lead to increased fat production. Gaines (36), using his composite figure, presents data concerning the coefficients for 939 cows making two records in the same herd, and 237 cows making two records in different herds. He concludes that the first eight monthly tests of each lactation may be used for genetic analysis. Giuliani (37), working with Brown Alpine and Friesian cows in Lombardy, mentions difficulties of comparing yields at different farms. He also gives records concerning the high yielders, including the progeny of the American-bred Holstein bull "Carnation Producer" which is being used in North Italy for the improvement of the existing types. Borini (38) deals similarly with the various breeds around Venice. Davis (39) has some interesting figures from Tasmania showing averages for different districts.

Proven sire

During the past 2 years there has not been the same spate of material regarding progeny testing. An anonymous writer (40) has produced an extremely interesting report on the method adopted in Holland for the progeny testing of bulls based on a system which has been in existence since 1910. Ever-increasing standards of performance are set, and a bull whose progeny meet the requirements earns the title "Preferent". A committee reports on such an animal, and this report is made available to breeders, together with a photograph of the bull and a description of the progeny, including a detailed account of the conformation and the inheritance of the shape of the udder, etc. Particular notice is taken of butterfat, and for this a graph is given on which points representing daughter-dam comparisons are plotted. Cows are grouped into nine butterfat percentage classes. There is no fixed standard for individual points of merit, type, milk, or butterfat. The system, while arbitrary and

perhaps artificial, appears to be of practical use. Altschuler & Suhanov (41) deal with the choice of dams for a mating campaign. They state that though progeny testing is one of the most fundamental factors in livestock improvement, it should not suppress all other breeding practices. The poorest yielders should be excluded from the test, while at the other end of the curve the best yielders should be mated with the best bulls in order to produce the required number of replacement bulls. They prefer considering the straight yields of the daughters without relation to the dam's production. They hold that progeny testing must continue, and that it is not sufficient to detect an improver, but that this should be continued from one generation to another, as only certain of the offspring of a proven sire will prove to be leaders and be capable of causing the further progress of the line. Lush & Shultz (42) deal with the first 355 dairy sires proved in Iowa. Forty-three per cent of all sires were proved with only five dam and daughter pairs. As usual there was considerable regression from the dams towards the average of the breed, so that a majority of the daughters out of cows producing less than 400 lb. were better than their dams: but as the dams' production rose above 400 lb. the percentage of daughters which were better than those dams decreased rapidly. There are included observations on management and environment. M'Candlish & Struthers (43) state that economy of production is of greater importance than maximum production, and describe the improvements which have occurred in Scotland. They conclude that there are few sires of real value, and that there is need for greater care in the selection of dairy bulls. A study of the male ancestry of the Ayrshire sires shows that 82 per cent of the bulls had one or more proven sires in their three nearest male ancestors.

In the meantime from the originator of the Mount Hope index comes a book on the subject. Prentice (44), in his opening chapters, deals with the historical aspect of cattle breeding, and deprecates the formalism to be found on the continent of Europe. He concludes that genealogies, type, and pedigree have failed sufficiently to improve the dairy qualities of our cattle, and if marked improvement is to be made, it must come from the progeny test. The final chapters deal almost entirely with the Mount Hope index which has now been simplified into a commercial form as follows: "If the average mature equivalent of the dam's production is 8000 lb. of milk, and the daughters' average mature equivalent is 10,000 lb. of milk, the bull is a 1200 lb. bull; if the average mature equivalent of the dam's production is 10,000 lb. of milk, and the daughters' average mature equivalent is 8000 lb. of milk, the bull is a 6000 lb. bull." The author rightly stresses the importance of the herd test where every cow in the herd is tested, but there are many statements with which other investigators will violently disagree. The subject is not studied comprehensively, and does not refer to the work of Edwards (45), who has tested very thoroughly the various bull indices on a comparable basis, and has shown that in practice the average of the daughters of a bull is as accurate as any other in estimating the yield of his future daughters.

Tropical

In the consideration of questions concerning the improvement of livestock in the Tropics insufficient attention has been paid to the question of the metabolic rates of different types of animals. Rhoad (46) has made a study of the influence of tropical temperatures upon the metabolic rate as indicated by rhythm of respiration of dairy cattle in Brazil. The high external temperatures and humidity influenced European and Indian cattle differently, thus indicating a species difference. As regards cross-breds the respiration curve approximates that of the European, though somewhat lower. The writer suggests that the loss of energy in dairy cattle as the result of high temperatures is, in a large way, responsible for the low production records of European

dairy cattle in the Tropics. In another paper(47) he describes these matters in greater detail in relation to the cattle of Brazil. Barbier(48) describes the milk yields of the cattle of Morocco and their improvement.

Hartley & Baker(49) make a comprehensive analysis of the milk of the cattle kept on the Government Farm near Zaria, N. Nigeria. Some evidence is offered to show that a change in butterfat content as regards morning and evening milking is directly due to the marked climatic differences between the two seasons, and that it cannot be controlled by altering the diet of the cattle.

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